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REVIEW

An expandable donor-free supply of functional hepatocytes for Toxicology

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The B-13 cell is a readily expandable rat pancreatic acinar-like cell that differentiates on simple plastic culture substrata into replicatively-senescent hepatocyte-like (B-13/H) cells in response to glucocorticoid exposure. B-13/H cells express a variety of liver-enriched and liver-specific genes, many at levels similar to hepatocytes in vivo. Furthermore, the B-13/H phenotype is maintained for at least several weeks in vitro, in contrast to normal hepatocytes which rapidly de-differentiate under the same simple - or even under more complex - culture conditions. The origin of the B-13 cell line and the current state of knowledge regarding differentiation to B-13/H cells are presented, followed by a review of recent advances in the use of B-13/H cells in a variety of toxicity endpoints. B-13 cells therefore offer Toxicologists a cost-effective and easy to use system to study a range of toxicologically-related questions. Dissecting the mechanism(s) regulating the formation of B-13/H cell may also increase the likelihood of engineering a human equivalent, providing Toxicologists with an expandable donor-free supply of functional rat and human hepatocytes, invaluable additions to the tool kit of in vitro toxicity tests.

Origin of the B-13 cell line

The AR42J-B13 (henceforth referred to as the B-13) cell line was sub-cloned from the rat adenocarcinoma AR42J cell line in the late 1970s. The AR42J cell line is an exocrine pancreatic adenocarcinoma cell line still used to the present day in basic exocrine pancreas research.[1-6] The AR42J cell line was derived from studies in which inbred Wistar/Lewis rats were treated with azaserine (O-diazoacetyl-L-serine).[7] Azaserine is a naturally occurring antibiotic first isolated from *Streptomyces fragilis*[8] that causes cytotoxicity in cells through at least 2 mechanisms:- inhibition of N-formylglycineamidine ribotide synthetase (PFAS, phosphoribosylformylglycinamidine synthase), which results in an inhibition of purine biosynthesis;[9] and through spontaneous decomposition to yield diazoacetate, which carboxymethylates DNA primarily on the O_6 and N_7 of guanine and the N_3 of adenine.[10-12] Azaserine is also an inhibitor of glucosamine-6-phosphate isomerase (GFPT1)[9] and is often used experimentally in studies on hexosamine biosynthesis.[13] A single intraperitoneal injection of 30 mg azaserine/kg body weight is capable of inducing adenoma and subsequently carcinoma in rats several months after exposure, primarily in the pancreas.[14] Accordingly, azaserine has been used in the past to generate experimental models of pancreatic cancer.[15-19] Why the pancreas is a target organ for tumours with azaserine however, remains unclear.

AR42J cells were isolated from tumour cells generated from the rat azaserine studies of Longnecker et al[7] and were transplanted back into untreated rats of the same strain by subcutaneous and intraperitoneal injections up to five times. The transplanted cells were reported to be capable of forming tumours at the site of injection and in lung and liver in some recipients.[7] The AR42J cell line generated was noted to express high levels of amylase and other pancreatic exocrine enzymes.[20] For summary, see Fig. 1.

The B-13 cell line was sub-cloned from the AR42J cell line by the Kojima lab in 1996 and was reported to differentiate into insulin-producing pancreatic endocrine β-like cells in vitro after treatment with hepatocyte growth factor (HGF) and activin A.[21] More recently, this effect has been shown to require expression in B-13 cells of transcription factors Ngn3 together with Nkx6.1 or MafA.[22]

In 2000, Shen et al observed that the B-13 cell underwent a profound alteration in phenotype from the pancreatic exocrine-like cell to an hepatocyte-like cell in response to glucocorticoid exposure.[23] In this study, a coordinated change in B-13

phenotype to an hepatocyte-like (B-13/H) cell - and not simply an occasional aberrant expression of an hepatocyte gene - appeared to be the case (see also Tables 1-3). A series of reports subsequent to this discovery essentially confirm these observations and demonstrate that B-13/H cells express a variety of genes that are hepatocyte-specific or hepatocyte-enriched, including expression of blood proteins, cytoskeletal proteins, metabolic enzymes, xenobiotic metabolising enzymes (both phase I and phase II) and transporter proteins (for details see Table 1-3).

Mechanisms regulating B-13 differentiation into hepatocyte-like cells

Progenitor nature of B-13 cells

The B-13 cell line readily proliferates in simple cell culture media such as Dulbecco's modified Eagle medium (DMEM) supplemented with serum (typically 10% (v/v)), with a doubling time of 30 +/- 7.3 hours.[46] The cells do not require any of the culture modifications often associated with stem cell maintenance (such as fibroblast feeders, matrigel or colony selection during sub-culture to select for progenitor characteristics). The cells will readily expand on plastic substrata and appear to have an altered - but stable - near tetraploid karyotype of male origin.[27] It is unknown for how long this karyotype has been stable, however B-13 cells have been repeatedly trans-differentiated into B-13/H cells for last 15 years, suggesting alterations at the genomic DNA level are very stable. B-13 cells do not grow in agar in vitro, and do not readily form tumours in SCID mice. However, B-13 cells are capable of selectively engrafting into the pancreas and liver after intravenous injection, and spontaneously forming hepatocyte-like cells in the liver.[27] B-13 cells therefore appear to retain some of the pathways that restrain dysregulated cell growth and differentiation. Were it not for their unique ability to differentiate into hepatocyte-like cells, B-13 cells might be considered no different from any other cell line.

The liver and pancreas are thought to contain progenitor cells localised in the ductal regions of the tissue (Fig. 2). Using the neural marker synaptophysin – which is expressed by B-13 cells and suppressed in B-13/H cells,[26] it is possible to identify putative progenitors in a limited number of both rat liver and pancreas ductal regions. The study of these progenitor cells in vitro is challenging since, in contrast to B-13 cells, the progenitor cells are difficult to purify and maintain as a pure population of progenitors. In the rat, liver progenitors (oval cells) are bi-potential and give rise to hepatocytes and biliary epithelial cells (cholangiocytes) in vivo.[85,86] In our hands, rat oval cells in culture rapidly generate biliary epithelial cells and/or fibroblasts (possibly through epithelial-mesenchymal trans-differentiation of biliary epithelial cells) and form a minority of the cell population within a day or two of isolation (Fig. 3).

The data to date suggest that the B-13 may be equivalent to a pancreatic or liver progenitor cell since it trans-differentiates from the B-13 acinar phenotype directly to the hepatocyte-like B-13/H phenotype. Using a B-13 cell line stably transfected with a green fluorescent protein (GFP) gene under control of a promoter (which is trans-activated in exocrine pancreas cells) Shen et al[23] showed that after exposure to glucocorticoid, cells could be identified that expressed both GFP and hepatocyte markers, suggesting that the mechanism of altered differentiation is likely a direct conversion from its "differentiated" pancreatic exocrine cell type directly to an hepatocyte, without reverting to a plastic progenitor-like intermediate). However, more recently, limited evidence suggests that B-13 cells may be directed to form a ductal (i.e. biliary-like) phenotype in response to glucocorticoid and EGF.[87] However, the identity of these cells is ambiguous, likely because the response lacks the quantitative trans-differentiation response seen with B-13/H cells and hepatocytes. This apparent plastic nature of B-13 cells may be reflective of the type of responses seen in a variety of cells in vitro, such as epithelial-mesenchymal transition (EMT), which commonly occur in vitro.[88,89] However, whether B-13 cells enter (or are capable under certain circumstances of entering) a transient plastic state prior to conversion to B-13/H cells remains uncertain.

Mechanism(s) mediating the formation of B-13/H cells

There is good evidence to suggest that the glucocorticoid receptor (GR) mediates the response to glucocorticoid in B-13 cells and initiates the process of trans-differentiation. The synthetic glucocorticoid dexamethasone is frequently employed in generating B-13/H cells because it is less rapidly metabolised than natural glucocorticoids and is more selective for the GR than many natural glucocorticoids.[29,30] However, B-13 cells respond similarly to a range other glucocorticoids and the response is inhibited by GR antagonists.[23,90] Other classes of steroids (e.g. oestrogens) fail to promote the trans-differentiation of B-13 cells into B-13/H cells[90] (see also Fig. 4) and although glucocorticoids also activate the mineralocorticoid receptor,[91] B-13 trans-differentiation to B-13/H cell does not occur in response to mineralocorticoids, nor is glucocorticoid-dependent trans-differentiation blocked by mineralocorticoid receptor antagonists.[92]

Previous studies over the years have reported the appearance of hepatocyte-like tissue in pancreatic exocrine tissue in vivo, in response to a variety of pathological conditions (Table 4). More recent observations have focussed on the effects of glucocorticoid on pancreatic exocrine tissue differentiation. These studies have shown that both rodent and human embryonic acinar cells can be converted into hepatocyte-like cells in response to glucocorticoid in vitro.[109,110] However, of fundamental importance from a practical point of view, the quantitative functionality of these pancreatic hepatocyte-like cells to hepatocytes from the liver was rarely made. When such comparisons were made, on a quantitative basis, it is arguable whether such cells could be defined as hepatocytes. However, this lack of quantitative comparability

may be a feature of the in vitro environment in which the cells were cultured, and does not indicate necessarily that the cells were not capable of forming functional hepatocytes in the correct environment (such as in vivo). This is supported by recent studies in both rodents and man.[24,90]

Using in vivo models, Wallace et al demonstrated that treatment with high doses of glucocorticoid to adult rats for just a few weeks is enough to convert occasional pancreatic acinar cells into cells that express liver-levels of CYP2E1.[24] In a transgenic mouse with high circulating endogenous circulating glucocorticoids and which showed many of the clinical features of Cushing's disease, significant liver-levels of hepatocyte gene expression could be observed in the pancreata of many mice by adulthood (21 weeks of age).[107] The normal pancreas is therefore capable of altering to an hepatocyte-like phenotype that shows comparable levels of hepatocyte gene expression in vivo when exposed to pathologically high levels of glucocorticoid. Interestingly, these data also show that such a phenomenon is not restricted to embryonic or foetal tissue. Recent investigations also indicate that pancreata from adult patients maintained on high levels of systemic glucocorticoid therapy also express hepatocyte levels of hepatocyte expressed genes,[90] suggesting that the observations in rodents are likely translatable to man.

Although it was clear from the first observations by Shen et al that the liver-enriched transcription factor CCAAT/enhancer-binding protein-β (CEBP-β) was a pivotal downstream mediator of B-13 trans-differentiation to B-13/H cells,[23] the signalling pathways that lead from glucocorticoid to the induction of this transcription factor in B-13 cells are only recently being determined. Wallace et al provide strong evidence to suggest that GR activation in B-13 cells leads to a robust induction of the serine/threonine kinase SGK1[92] and that this kinase, at least in part, phosphorylates the Wnt signalling protein messenger β-catenin as a component of the mechanism that precedes induction of transcription factors such as CEBP-β (see also Fig. 5c for schematic diagram of proposed proximal mechanism of trans-differentiation).[48,29]

The physiological function of SGK1 in vivo is in the regulation of sodium salt re-absorption by the kidney. The kinase functions to mediate mineralocorticoid stimulation of renal sodium channel (ENaC) activity and anti-natriuresis through its phosphorylation of the ubiquitin ligase Nedd4-2, which leads to a block in epithelial ENaC ubiquitylation, a reduction in ENaC endocytosis and increased renal tubular epithelial sodium transport into the cell.[111] SGK1 mediates a stress response to low salt levels, promoting salt re-absorption. Accordingly, knockout of the Sgk1 gene in mice has no overt effect on mice unless the mice are forced onto a low salt intake.[112,113] To date, a role for SGK1 in cell differentiation and/or tissue development – with the exception of B-13 cells - has not been identified. However, a limited microarray study that compared the levels of approximately 5000 transcripts in B-13 and B-13/H cells identified SGK1 as the highest induced transcript in response to glucocorticoid treatment [supplementary data to Wallace et al[24]]. Subsequent studies demonstrated that expression of either of 2 human SGK1 variants (but not the "wild type" variant A) alone, drove B-13 trans-differentiation into B-13/H cells (see also Fig. 5).[92] The SGK1 variants are identical with the exception of alternative N-terminal domains. These differentiation-promoting SGK1 variants are also induced in both mouse and human pancreatic acinar hepatic tissue or cells after exposure to high glucocorticoid.[107,90] Using recombinant SGK1 and β-catenin, it was shown that SGK1 was able to phosphorylate β-catenin on the residues that target β-catenin for ubiquitination and degradation,[92] a process that would be expected to down-regulate the Wnt signalling pathway. A Wnt signalling-responsive promoter-reporter gene construct suggested that Wnt signalling activity was highly active in B-13 cells.[48] Inhibition of Wnt signalling (using the inhibitor quercetin or through over-expression of a mutant β-catenin [pt-Xβ-cat]) or siRNA to knockdown β-catenin expression) alone resulted in Cebp-β induction and trans-differentiation of B-13 cells into B-13/H cells.[48] It is likely therefore, that specific Sgk1 variants provide the link between GR activation and the Wnt signalling pathway, and that this crosstalk lies upstream of Cebp-β induction and trans-differentiation. In normal cells, this cross talk is unlikely to be operational and is only relevant in pathological situations such as chronic hyper-elevated glucocorticoid levels. However, the Sgk1 cross talk with Wnt signalling may be amplified in some way in B-13 cells (e.g. through an over-induction and/or sustained elevation in SGK1 expression and/or a constitutively active PI3 kinase signalling pathway) such that the crosstalk mechanism is responsive in B-13 cells at physiologically normal concentrations of glucocorticoid.

Notable features of B-13/H cell formation

There are several features of the trans-differentiation of B-13 cells into B-13/H cells that are striking, particularly in the context of current attempts to generate functional hepatocytes from embryonic stem cells or induced pluripotent stem cells for use in hepatology or toxicology research.

Treatment with glucocorticoid is sufficient to promote the trans-differentiation of B-13 cells into B-13/H cells. This contrasts markedly with the range of different growth factors and other poorly defined additions (e.g. matrigel, fibroblast feeder layers) that are required or often used when ESCs or iPSCs are directed to differentiate into hepatocyte-like cells in vitro. Recombinant growth factors are also relatively expensive to produce and purify and are subject to variable functional activity, whereas glucocorticoids are inexpensive and relatively simple, stable chemicals.

A major physiological function of glucocorticoids is as a regulator of general metabolism. Glucocorticoids are synthesised by the adrenals and the levels secreted into the blood are controlled by the hypothalamus-pituitary-adrenal (HPA) axis.[114] Glucocorticoids are stress hormones and high levels promote gluconeogenesis, lipolysis and protein and amino acid degradation.[114] Glucocorticoids also promote

diuresis and have a general immunosuppressive (most notably, anti-inflammatory) effect, a feature that is often employed for therapeutic reasons.[115] However, glucocorticoids also have a developmental role, at least within the foetus. Circulating levels of glucocorticoid are low in the embryo and foetus (placental enzymes such as 11β-hydroxysteroid dehydrogenase [HSD2] metabolise glucocorticoids to inactive products and foetal levels only rise as the foetal HPA axis develops).[116,140] This rise in circulating foetal glucocorticoid probably plays a major role in cell differentiation and tissue maturation, most notably in the lung, since premature babies are successfully treated with glucocorticoids to promote lung maturation.[117]

A biologically relevant GR activating concentration of glucocorticoids (typically 10^{-8} M) is sufficient to promote the trans-differentiation of B-13 cells into B-13/H cells. The affinity of the rat GR for dexamethasone is in the 10^{-9} M range (K_D = 2.3nM[118]) and typically, 10^{-8} M dexamethasone is employed to robustly activate the GR in cells in vitro. The GR is expressed in B-13 cells, is transcriptionally functional and its activity and apparent ability to drive B-13/H formation at 10nM dexamethasone is blocked by GR antagonists.[90,119] In primary pancreatic rodent and human acinar cell cultures, significantly higher (10^{-6} to 10^{-5} M) concentrations are required (and produce a quantitatively much reduced trans-differentiation to hepatocyte-like phenotype).[87,90] The reason(s) for this are unclear. Although there is another receptor protein for dexamethasone in the rat with a lower affinity for dexamethasone (K_D = 59nM[118]), the antagonist RU486 does not block dexamethasone binding to this protein, whereas RU486 blocks primary pancreatic rodent and human acinar cell trans-differentiation to hepatocyte-like cells.[23,92] These observations therefore suggest that the activated GR may be hyper-functional in B-13 cells, although the reason(s) for this remain to be elucidated.

Exposure of B-13 cells to glucocorticoid converts the majority of the cells into a non-replicative B-13/H phenotype, with often less that 5% of the cells remaining in a B-13 phenotype. From a practical point of view, a striking feature of hepatocyte-like cell generation from B-13 cells is the near complete trans-differentiation of B-13 cells to B-13/H cells in response to glucocorticoid. It is therefore possible to generate a near pure population of hepatocyte-like cells with relative ease. This contrasts with the potential for ESCs and iPSCs to differentiate into a variety of cells, although protocols are beginning to address this problem and hepatocyte purity has increased from early reports.

Although the liver has the capacity to regenerate in vivo through mitosis of existing hepatocytes in vivo, hepatocytes in culture do not readily undergo mitosis in vitro.[119] Through additions of growth factors, low seeding densities etc, it is possible to stimulate DNA synthesis in cultured hepatocytes, but hepatocytes rarely complete the cell cycle in vitro and therefore, the cells are not readily expanded in vitro in the way that replicative cells lines may be expanded.[119,120] In this respect, it is possible that the de-differentiation of hepatocytes when they are cultured under conditions designed to promote growth, has the effect of pushing them into the cell cycle from which they cannot emerge.[121] In contrast, avoiding this regenerative-like response through culturing cells in sandwich cultures of extracellular matrix and/or 3D matrices may explain their superiority in maintaining hepatic function in culture [51-53].[122-124]

B-13 cells are resistant to differentiation into other cell types in response to typical differentiating factors such as soluble growth factors and cytokines. In the liver, the progenitor cell (termed oval cell in rodents) is bi-potential and capable of differentiating into either hepatocytes or biliary epithelial cells.[85,86] It is thought that oval cells only give rise to significant numbers of hepatocytes in liver regeneration when hepatocytes are prevented from undergoing mitosis themselves.[85,86] If the B-13 cell is derived or markedly related in function to a pancreatic/hepatic progenitor, then one might expect that the B-13 cell be capable of differentiating into other pancreatic and/or hepatic cell types.

B-13 cells have been directed toward a pancreatic β cell phenotype although there appears to be a requirement for ectopic over-expression of specific transcription factors.[21,22,125] Treating B-13 cells with glucocorticoid and then EGF also promotes a ductal-like phenotype.[87]

Since it is arguably the case that many cell types can be forced to alter their phenotype into other cell types if the appropriate transcription factors are over-expressed (often referred to as direct lineage conversion[126]:- the induction of pluripotency by c-Myc, Klf4, Oct4 and Sox2[127,128] being effectively direct linear conversion to pluripotent stem cells / indirect linage conversion to other mature cells), then it may not be surprising that B-13 cells can be directed into phenotypes other than hepatocytes when given the required non-hormonal signals, if sufficiently potent. However, in all cases so far, the quantitative comparable functional differentiation of B-13 cells into other cell types in response to hormonal factors alone, does not compare with the robust response seen with respect to glucocorticoid and hepatocyte-like cell formation. Thus, B-13 cells resist differentiation into other cell types in response to typical differentiating factors such as soluble growth factors and cytokines.

B-13/H cells are quantitatively similarly functional to freshly isolated rat hepatocytes. As outlined above, a marked feature of B-13/H formation from B-13 cells is their quantitative comparability to primary rat hepatocytes. This feature is relevant in particular, when considering in vitro cell models for studying toxicity mechanisms and potentially using the model to screen for drug and chemical toxicity.

Most chemical toxicities (both cytotoxic and genotoxic) in man are the result of metabolic activation in a target tissue(s) because exposure to directly cytotoxic reactive chemicals are normally avoided.[129] A major driver of activation pathways is the level of expression of gene products associated with pro-toxin activation. In simplistic terms, expression of genes associated with metabolic activation are required to be expressed at levels at least approximating to those found in the target organ(s) for the cells to be a realistic in vitro model for

toxicity assessment. This remains only an initial requirement since, additional layers of complexity would need to be taken into account (e.g. toxicokinetics, protective pathways etc) before such in vitro models could be used to predict in vivo toxicity.

Conclusions

The B-13 cell line provides an expandable donor-free supply of functional rat hepatocyte-like cells that can be readily used without a requirement for expertise found outside the average toxicology laboratory. There remain some limitations to B-13/H cells, such as the fact that they are rat cells and not human, and that there are limitations in the expression of some transporters such as NTCP. However, given their ease of use and cost effectiveness, on balance B-13 cells have clear value in toxicity testing. Loss of some genes, such as CYP1A2, can be overcome through ectopic expression (e.g. the human gene). Furthermore, understanding the unique features of this cell line, when applied to human stem cell-derived hepatocytes, may also be essential in the drive toward generating more functional and stable hepatocytes in vitro.

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Notes and references

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Abbreviations:AdV, adenovirus; CPSI, carbamyl phosphate synthase ; CYP, cytochrome P450; CK-19, cytokeratin 19 (commonly used ductal cell marker); DAPI, 2-(4-Amidinophenyl)-6-indolecarbamidine dihydrochloride (a fluorescent DNA stain); DEX, dexamethasone; E2 17β oestradiol; DMEM, Dulbecco's modified Eagle medium; EMT, epithelial-mesenchymal transition; ENaC, renal sodium channel; ER, oestrogen receptor [rERα, rat oestrogen receptor α; rERβ rat oestrogen receptor β]; GAPDH, glyceraldehyde phosphate dehydrogenase; GFP, green fluorescent protein; GSK, GSK 650394 (an SGK1 inhibitor); ICI, ICI182780 (an ER antagonist); NTCP, Na^+-taurocholate cotransporting polypeptide; PMP70, 70-kDa peroxisomal membrane protein (ABCD3); PPARα, peroxisomal proliferator activated receptor α; RT-PCR, reverse transcription, polymerase chain reaction; qRT-PCR, quantitative RT-PCR.

References

1 Z. Li, M. Lu, J. Chu, X. Qiao, X. Meng, B, Sun, W. Zhang and D. Xue, Early proteome analysis of rat pancreatic acinar AR42J cells treated with taurolithocholic acid 3-sulfate, *Pancreatology* 2012, **12,** 248-56.
2 L. Garcia-Sanchez, P. Santofimia-Castaño, A. Miro-Moran, J. A. Tapia, G. M. Salido and A. Gonzalez, Resveratrol mobilizes Ca2+ from intracellular stores and induces c-Jun N-terminal kinase activation in tumoral AR42J cells, *Mol Cell Biochem* 2012, **362**, 15-23.
3 H. Sebai, E. Ristorcelli, V. Sbarra, S. Hovsepian, G. Fayet, E. Aouani and D. Lombardo, Protective effect of resveratrol against LPS-induced extracellular lipoperoxidation in AR42J cells partly via a Myd88-dependent signaling pathway, *Arch Biochem Biophys* 2010, **495**, 56-61.
4 E. Twait, D. E. Williard and I. Samuel, Dominant negative p38 mitogen-activated protein kinase expression inhibits NF-kappaB activation in AR42J cells, *Pancreatology* 2010, **10**, 119-28.
5 D. Xue, W. Zhang, T. Liang, S. Zhao, B. Sun and D. Sun, Effects of arsenic trioxide on the cerulein-induced AR42J cells and its gene regulation, *Pancreas* 2009, **38**, e183-9.
6 J. Chu, H. Ji, M. Lu, Z. Li, X. Qiao, B Sun, W. Zhang and D. Xue, Proteomic analysis of apoptotic and oncotic pancreatic acinar AR42J cells treated with caerulein, *Mol Cell Biochem* 2013, **382**, 1-17.
7 D. S. Longnecker, H. S. Lilja, J. French, E. Kuhlmann and W. Noll, Transplantation of azaserine-induced carcinomas of pancreas in rats, *Cancer Lett* 1979, **7**, 197–202.
8 J. Ehrlich, L. E. Anderson, G. L. Coffey, A. B. Hillegas, M. P. Knudsen, H. J. Koepsell, D. L. Kohberger and J. E. Oyaass, Antibiotic studies of azaserine, *Nature* 1954, **173**, 72.
9 S. D. Lyons, M. E. Sant and R. I. Christopherson, Cytotoxic mechanisms of glutamine antagonists in mouse L1210 leukemia, *J Biol Chem* 1990 **265**, 11377-81.
10 J. Zurlo, T. J. Curphey, R. Hiley and D. S. Longnecker, Identification of 7-carboxymethylguanine in DNA from pancreatic acinar cells exposed to azaserine, *Cancer Res* 1982, **42**, 1286-8.
11 K. L. Harrison, N. Fairhurst, B. C. Challis and D. E. Shuker, Synthesis, characterization, and immunochemical detection of O6-(carboxymethyl)-2'-deoxyguanosine: a DNA adduct formed by nitrosated glycine derivatives, *Chem Res Toxicol* 1997, **10**, 652-9.
12 M. O'Driscoll, P. Macpherson, Y. Z. Xu and P. Karran, The cytotoxicity of DNA carboxymethylation and methylation by the model carboxymethylating agent azaserine in human cells, *Carcinogenesis* 1999, **20**, 1855-62.
13 J. Kim, G. Song, G. Wu and F. W. Bazer, Functional roles of fructose, *Proc Natl Acad Sci U S A* 2012, **109**, E1619-28.
14 C. Löser, E. Stüber and U. R. Fölsch, Dissimilar effect of the carcinogenic agent azaserine on pancreatic and hepatic polyamine metabolism in rats, *Pancreas* 1995, **10**, 44-52.
15 M. J. Appel and R. A. Woutersen, Effects of a diet high in fish oil (MaxEPA) on the formation of micronucleated erythrocytes in blood and on the number of atypical acinar cell foci Induced in rat pancreas by azaserine, *Nutr Cancer* 2003, **47**, 57-61.

16 M. J. Appel and R. A. Woutersen, Effects of dietary beta-carotene and selenium on initiation and promotion of pancreatic carcinogenesis in azaserine-treated rats, *Carcinogenesis* 1996, **17**, 1411-6.
17 R. A. Woutersen, A. Van Garderen-Hoetmer, J. Bax and E. Scherer, Modulation of putative preneoplastic foci in exocrine pancreas of rats and hamsters. Interaction ofdietary fat and coffee, *Dig Dis Sci* 1989, **34**, 789-96.
18 Y. Takiyama, R. A. Woutersen, and P. M. Pour PM. Ulex Europaeus-I: a marker for differentiation of (pre)cancerous lesions induced in the rat pancreas by azaserine, *Carcinogenesis* 1988, **9**, 2087-92.
19 J. Bax, A. W. Feringa, A. van Garderen-Hoetmer, R. A. Woutersen and E. Scherer, Adenosine triphosphatase, a new marker for the differentiation of putative precancerous foci induced in rat pancreas by azaserine, *Carcinogenesis* 1986, **7**, 457-62.
20 N. W. Jessop and R. J. Hay, Characteristics of two rat pancreatic exocrine cell lines derived from transplantable tumours, *In Vitro* 1980, **16**, 212.
21 H. Mashima, H. Shibata, T. Mine and I. Kojima I, Formation of insulin-producing cells from pancreatic acinar AR42J cells by hepatocyte growth factor, *Endocrinology* 1996, **137**, 3969-76.
22 T. Ogihara, Y. Fujitani, T. Uchida, R. Kanno, J. B. Choi, T. Hirose, R. Kawamori and H. Watada, Combined expression of transcription factors induces AR42J-B13 cells to differentiate into insulin-producing cells, *Endocr J* 2008, **55**, 691-8.
23 C. N. Shen, J. M. Slack and D. Tosh, Molecular basis of transdifferentiation of pancreas to liver, *Nat Cell Biol* 2000, **2**, 879-87.
24 K. Wallace K, C. J. Marek, R. A. Currie and M. C. Wright, Exocrine pancreas trans-differentiation to hepatocytes--a physiological response to elevated glucocorticoid in vivo, *J Steroid Biochem Mol Biol* 2009, **116**, 76-85.
25 J. K. Kurash, C. N. Shen and D. Tosh, Induction and regulation of acute phase proteins in transdifferentiated hepatocytes, *Exp Cell Res* 2004, **292**, 342-58.
26 Z. D. Burke, C. N. Shen, K. L. Ralphs and D. Tosh, Characterization of liver function in transdifferentiated hepatocytes, *J Cell Physiol* 2006, **206**, 147-59.
27 E. A. Fairhall, M. A. Charles, K. Wallace, C. J. Schwab, C. J. Harrison, M. Richter, S. A. Hoffmann, K. A. Charlton, K. Zeilinger and M. C. Wright, The B-13 hepatocyte progenitor cell resists pluripotency induction and differentiation to non-hepatocyte cells, *Toxicology Research* 2013, **2**, 308 - 320.
28 K. Wallace, E. A. Fairhall, K. A. Charlton and M. C. Wright, AR42J-B-13 cell: an expandable progenitor to generate an unlimited supply of functional hepatocytes, *Toxicology* 2010, **278**, 277-87.
29 D. Tosh, C. N. Shen and J. M. Slack, Differentiated properties of hepatocytes induced from pancreatic cells, *Hepatology* 2002; **36**, 534-43.
30 Z. Filip, K. Jan, S. Vendula, K. Z. Jana, M. Kamil and K. Kamil, Albumin and α1-acid glycoprotein: old acquaintances, *Expert Opin Drug Metab Toxicol* 2013, **9**, 943-54.
31 K. Gkouvatsos, G. Papanikolaou and K. Pantopoulos, Regulation of iron transport and the role of transferrin, *Biochim Biophys Acta* 2012, **1820**, 188-202.
32 K. Yamauchi and A. Ishihara, Evolutionary changes to transthyretin: developmentally regulated and tissue-specific gene expression, *FEBS J* 2009, **276**, 5357-66.
33 S. M. Janciauskiene, R. Bals, R. Koczulla, C. Vogelmeier, T Köhnlein and T. Welte, The discovery of α1-antitrypsin and its role in health and disease, *Respir Med* 2011, **105**, 1129-39.
34 P. B. Armstrong and J. P. Quigley, Alpha2-macroglobulin: an evolutionarily conserved arm of the innate immune system, *Dev Comp Immunol* 1999; **23**, 375-90.
35 A. I. Alayash, Haptoglobin: old protein with new functions, *Clin Chim Acta* 2011, **412**, 493-8.
36 G. J. Mizejewski, Biological roles of alpha-fetoprotein during pregnancy and perinatal development, *Exp Biol Med (Maywood)* 2004, **229**, 439-63.
37 A. B. Wang, D. P. Liu and C. C. Liang, Regulation of human apolipoprotein B gene expression at multiple levels, *Exp Cell Res* 2003, **290**, 1-12.
38 P. Strnad, C. Stumptner, K. Zatloukal and H. Denk, Intermediate filament cytoskeleton of the liver in health and disease, *Histochem Cell Biol* 2008, **129**, 735-49.
39 J. C. Hutton and R. M. O'Brien, Glucose-6-phosphatase catalytic subunit gene family, *J Biol Chem* 2009, **284**, 29241-5.
40 J. B. Dietrich, Tyrosine aminotransferase: a transaminase among others? *Cell Mol Biol* 1992, **38**, 95-114.
41 S. C. Nagamani, A. Erez and B. Lee, Argininosuccinate lyase deficiency, *Genet Med* 2012, **14**, 501-7.
42 Z. D. Burke, K. R. Reed, T. J. Phesse, O. J. Sansom, A. R. Clarke and D. Tosh, Liver zonation occurs through a beta-catenin-dependent, c-Myc-independent mechanism, *Gastroenterology* 2009, **136**, 2316-2324.
43 J. L. Deignan, S. D. Cederbaum and W. W. Grody, Contrasting features of urea cycle disorders in human patients and knockout mouse models, *Mol Genet Metab* 2008, **93**, 7-14.
44 S. Lenzen, A fresh view of glycolysis and glucokinase regulation: history and current status, *J Biol Chem* 2014, **289**, 12189-94.
45 C. J. Marek, G. A. Cameron, L. J. Elrick, G. M. Hawksworth and M. C. Wright, Generation of hepatocytes expressing functional cytochromes P450 from a pancreatic progenitor cell line in vitro, *Biochem J* 2003, **370**, 763-9.
46 P. M. Probert, G. W. Chung, S. J. Cockell, L. Agius, P. Mosesso, S. A. White, F. Oakley, C. D. Brown and M. C. Wright, Utility of B-13 progenitor-derived hepatocytes in hepatotoxicity and genotoxicity studies, *Toxicol Sci* 2014, **137**, 350-70.
47 P. M. Probert, In: *Refining and replacing models of hepatocytes and periportal fibrosis*. Thesis 2014. Available at https://theses.ncl.ac.uk/dspace/
48 K. Wallace, C. J. Marek, S. Hoppler and M. C. Wright, Glucocorticoid-dependent transdifferentiation of pancreatic progenitor cells into hepatocytes is dependent on transient suppression of WNT signalling, *J Cell Sci* 2010, **123**, 2103-10.
49 T. Iyanagi, C. Xia and J. J. Kim, NADPH-cytochrome P450 oxidoreductase: prototypic member of the diflavin reductase family, *Arch Biochem Biophys* 2012, **528**, 72-89.
50 F. P. Guengerich, Cytochrome p450 and chemical toxicology, *Chem Res Toxicol* 2008, **21**, 70-83.

51 T. D. Bradshaw and D. R. Bell, Relevance of the aryl hydrocarbon receptor (AhR) for clinical toxicology, *Clin Toxicol (Phila)* 2009, **47**, 632-42.
52 Y. E. Timsit and M. Negishi, CAR and PXR: the xenobiotic-sensing receptors, *Steroids* 2007, **72**, 231-46.
53 K. Shean and A. J. Paine, Immunochemical quantification of cytochrome P450IA and IIB subfamilies in the livers of metyrapone-treated rats. Relevance to the ability of metyrapone to prevent the loss of cytochrome P-450 in rat hepatocyte culture, *Biochem J* 1990, **267**, 715-9.
54 A. K. Agrawal and B. H. Shapiro, Constitutive and inducible hepatic cytochrome P450 isoforms in senescent male and female rats and response to low-dose phenobarbital, *Drug Metab Dispos* 2003, **31**, 612-9.
55 D. J. Waxman and M. G. Holloway, Sex differences in the expression of hepatic drugmetabolizing enzymes, *Mol Pharmacol* 2009, **76**, 215-28.
56 V. Wauthier and D. J. Waxman, Sex-specific early growth hormone response genes in rat liver, *Mol Endocrinol*. 2008, **22**, 1962-74.
57 M. C. Wright, R. J. Edwards, M. Pimenta, V. Ribeiro, G. S. Ratra, M. C. Lechner and A. J. Paine, Developmental changes in the constitutive and inducible expression of cytochrome P450 3A2, *Biochem Pharmacol* 1997, **54**, 841-6.
58 S. Green, PPAR: a mediator of peroxisome proliferator action, *Mutat Res* 1995, **333**, 101-9.
59 T. Matsubara, F. Li and F. J. Gonzalez, FXR signaling in the enterohepatic system, *Mol Cell Endocrinol* 2013, **368**, 17-29.
60 M. O. James and S. Ambadapadi, Interactions of cytosolic sulfotransferases with xenobiotics, *Drug Metab Rev* 2013, **45**, 401-14.
61 A. Rowland, J. O. Miners and P. I. Mackenzie, The UDP-glucuronosyltransferases: their role in drug metabolism and detoxification, *Int J Biochem Cell Biol* 2013, **45**, 1121-32.
62 M. Kishi, Y. Emi, M. Sakaguchi, S. Ikushiro and T Iyanagi, Ontogenic isoform switching of UDP-glucuronosyltransferase family 1 in rat liver, *Biochem Biophys Res Commun* 2008, **377**, 815-9.
63 M. K. Shelby, N. J. Cherrington, N. R. Vansell and C. D. Klaassen, Tissue mRNA expression of the rat UDP-glucuronosyltransferase gene family, *Drug Metab Dispos* 2003, **31**, 326-33.
64 C. D. King, G. R. Rios, M. D. Green, P. I. MacKenzie and T. R. Tephly, Comparison of stably expressed rat UGT1.1 and UGT2B1 in the glucuronidation of opioid compounds, *Drug Metab Dispos* 1997, **25**, 251-5.
65 P. G. Board and D. Menon, Glutathione transferases, regulators of cellular metabolism and physiology, *Biochim Biophys Acta* 2013, **1830**, 3267-88.
66. K. Yang, K. Köck, A. Sedykh, A. Tropsha and K. L. Brouwer KL, An updated review on drug-induced cholestasis: mechanisms and investigation of physicochemical properties and pharmacokinetic parameters, *J Pharm Sci* 2013, **102**, 3037-57.
67 T. L. Marion, E. M. Leslie and K. L. Brouwer, Use of sandwich-cultured hepatocytes to evaluate impaired bile acid transport as a mechanism of drug-induced hepatotoxicity, *Mol Pharm* 2007, **4**, 911-8.
68 H. Glaeser, Importance of P-glycoprotein for drug-drug interactions, *Handb Exp Pharmacol* 2011, **201**, 285-97.
69 E. Gonzales, A. Davit-Spraul, C. Baussan, C. Buffet, M. Maurice and E. Jacquemin, Liver diseases related to MDR3 (ABCB4) gene deficiency, *Front Biosci (Landmark Ed)* 2009, **14**, 4242-56.
70 S. M. He, R. Li, J. R. Kanwar and S. F. Zhou, Structural and functional properties of human multidrug resistance protein 1 (MRP1/ABCC1), *Curr Med Chem* 2011, **18**, 439-81.
71 D. Keppler, Multidrug resistance proteins (MRPs, ABCCs): importance for pathophysiology and drug therapy, *Handb Exp Pharmacol* 2011, **201**, 299-323.
72 K. Jemnitz, K. Heredi-Szabo, J. Janossy, E. Ioja, L. Vereczkey and P. Krajcsi, ABCC2/Abcc2: a multispecific transporter with dominant excretory functions, *Drug Metab Rev* 2010, **42**, 402-36.
73 P. Borst, C. de Wolf and K. van de Wetering, Multidrug resistance-associated proteins 3, 4, and 5, *Pflugers Arch* 2007, **453**, 661-73.
74 P. R. Wielinga, I. van der Heijden, G. Reid, J. H. Beijnen, J. Wijnholds and P. Borst, Characterization of the MRP4- and MRP5-mediated transport of cyclic nucleotides from intact cells, *J Biol Chem* 2003, **278**, 17664-71.
75 G. Reid, P. Wielinga, N. Zelcer, M. De Haas, L. Van Deemter, J. Wijnholds, J. Balzarini and P. Borst, Characterization of the transport of nucleoside analog drugs by the human multidrug resistance proteins MRP4 and MRP5, *Mol Pharmacol* 2003, **63**, 1094-103.
76 A. Váradi, Z. Szabó, V. Pomozi, H. de Boussac, K. Fülöp and T Arányi, ABCC6 as a target in pseudoxanthoma elasticum, *Curr Drug Targets* 2011, **12**, 671-82.
77 K. Natarajan, Y. Xie, M. R. Baer and D. D. Ross, Role of breast cancer resistance protein (BCRP/ABCG2) in cancer drug resistance, *Biochem Pharmacol* 2012, **83**, 1084-103.
78 B. Stieger, The role of the sodium-taurocholate cotransporting polypeptide (NTCP) and of the bile salt export pump (BSEP) in physiology and pathophysiology of bile formation, *Handb Exp Pharmacol* 2011, **201**, 205-59.
79 P. M. Probert and M. C. Wright, unpublished observations.
80 E. Lozano, E. Herraez, O. Briz, V. S. Robledo, J. Hernandez-Iglesias, A. Gonzalez-Hernandez and J. J. Marin, Role of the plasma membrane transporter of organic cations OCT1 and its genetic variants in modern liver pharmacology, *Biomed Res Int* 2013, **2013**, 692071.
81 W. K. Lee, N. A. Wolff and F. Thévenod F, Organic cation transporters: physiology, toxicology and special focus on ethidium as a novel substrate, *Curr Drug Metab* 2009, **10**, 617-31.
82 G. Burckhardt, Drug transport by Organic Anion Transporters (OATs), *Pharmacol Ther* 2012, **136**, 106-30.
83 S. Choudhuri, N. J. Cherrington, N. Li, C. D. Klaassen, Constitutive expression of various xenobiotic and endobiotic transporter mRNAs in the choroid plexus of rats, *Drug Metab Dispos* 2003, **31**, 1337-45.
84 T. Nishio, H. Adachi, R. Nakagomi, T. Tokui, E. Sato, M. Tanemoto, K. Fujiwara, M. Okabe, T. Onogawa, T. Suzuki, D. Nakai, K. Shiiba, M. Suzuki, H. Ohtani, Y. Kondo, M. Unno, S. Ito, K. Iinuma, K. Nunoki, S. Matsuno and T. Abe. Molecular identification of a rat novel organic anion transporter moat1, which transports prostaglandin D(2), leukotriene C(4), and taurocholate, *Biochem Biophys Res Commun* 2000, **275**, 831-8.
85 L. Erker and M. Grompe, Signaling networks in hepatic oval cell activation, *Stem Cell Res* 2007, **1**, 90-102.

86 L. Boulter, O. Govaere, T. G. Bird, S. Radulescu, P. Ramachandran, A. Pellicoro, R. A. Ridgway, S. S. Seo, B. Spee, N. Van Rooijen, O. J. Sansom, J. P. Iredale, S. Lowell, T. Roskams and S. J. Forbes, Macrophage-derived Wnt opposes Notch signaling to specify hepatic progenitor cell fate in chronic liver disease, *Nat Med* 2012, **18**, 572-9.

87 A. Al-Adsani, Z. D. Burke, D. Eberhard, K. L. Lawrence, C. N. Shen, A. K. Rustgi, H. Sakaue, J. M. Farrant and D. Tosh, Dexamethasone treatment induces the reprogramming of pancreatic acinar cells to hepatocytes and ductal cells, *PLoS One* 2010, **5**, e13650.

88 M. Pinzani, Epithelial-mesenchymal transition in chronic liver disease: fibrogenesis or escape from death? *J Hepatol* 2011, **55**, 459-65.

89 G. Xie and A. M. Diehl, Evidence for and against epithelial-to-mesenchymal transition in the liver, *Am J Physiol Gastrointest Liver Physiol* 2013, **305**, G881-90.

90 E. A. Fairhall, K. Wallace, S. A. White, G. C. Huang, J. A. Shaw, S. C. Wright, K. A. Charlton, A. D. Burt and M. C. Wright, Adult human exocrine pancreas differentiation to hepatocytes – potential source of a human hepatocyte progenitor for use in toxicology research, *Toxicology Research* 2013, **2**, 80 – 87.

91 J. R. Seckl and B. R. Walker, Minireview: 11beta-hydroxysteroid dehydrogenase type 1- a tissue-specific amplifier of glucocorticoid action, *Endocrinology* 2001, **142**, 1371-6.

92 K. Wallace, Q. Long, E. A. Fairhall, K. A. Charlton and M. C. Wright, Serine/threonine protein kinase SGK1 in glucocorticoid-dependent transdifferentiation of pancreatic acinar cells to hepatocytes, *J Cell Sci* 2011, **124**, 405-13.

93 D. G. Scarpelli, M. S. Rao, V. Subbarao and M. Beversluis, Regeneration of Syrian golden hamster pancreas and covalent binding of N-nitroso-2,6-[3H]dimethylmorpholine, *Cancer Res* 1981, **41**, 1051-7.

94 M. S. Rao, M. K. Reddy, J. K. Reddy and D. G. Scarpelli, Response of chemically induced hepatocytelike cells in hamster pancreas to methyl clofenapate, a peroxisome proliferator, *J Cell Biol* 1982, **95**, 50-6

95 J. K. Reddy, M. S. Rao, S. A. Qureshi, M. K. Reddy, D. G. Scarpelli and N. D. Lalwani, Induction and origin of hepatocytes in rat pancreas, *J Cell Biol* 1984, **98**, 2082-90.

96 M. S. Rao, V. Subbarao and M. K. Reddy, Induction of hepatocytes in the pancreas of copper-depleted rats following copper repletion, *Cell Differ* 1986, **18**, 109-17.

97 N. Usuda, J. K. Reddy, T. Hashimoto and M. S. Rao, Immunocytochemical localization of liver-specific proteins in pancreatic hepatocytes of rat, *Eur J Cell Biol* 1988, **46**, 299-306.

98 M. S. Rao, R. S. Dwivedi, V. Subbarao, M. I. Usman, D. G. Scarpelli, M. R. Nemali, A. Yeldandi, S. Thangada, S. Kumar and J. K. Reddy, Almost total conversion of pancreas to liver in the adult rat: a reliable model to study transdifferentiation, *Biochem Biophys Res Commun* 1988, **156**, 131-6.

99 N. Konishi, J. M. Ward and M. P. Waalkes, Pancreatic hepatocytes in Fischer and Wistar rats induced by repeated injections of cadmium chloride, *Toxicol Appl Pharmacol* 1990, **104**, 149-56.

100 M. S. Rao, V. Subbarao and D. G. Scarpelli, Development of hepatocytes in the pancreas of hamsters treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin, *J Toxicol Environ Health* 1988, **25**, 201-5.

101 R. S. Dwivedi, A. V. Yeldandi, V. Subbarao, P. Feigelson, A. K. Roy, J. K. Reddy and M. S. Rao, Androgen regulated expression of the alpha 2u-globulin gene in pancreatic hepatocytes of rat, *J Cell Biol* 1990, **110**, 263-7.

102 A. V. Yeldandi, X. D. Tan, R. S. Dwivedi, V. Subbarao, D. D. Smith Jr, D. G. Scarpelli, M. S. Rao and J. K. Reddy, Coexpression of glutamine synthetase and carbamoylphosphate synthase I genes in pancreatic hepatocytes of rat, *Proc Natl Acad Sci U S A* 1990, **87**, 881-5.

103 M. S. Rao, V. Subbarao, K. Sato and J. K. Reddy, Alterations of pancreatic hepatocytes in rats exposed to carcinogens, *Am J Pathol* 1991, **139**, 1111-7.

104 M. S. Rao, M. Yukawa, M. Omori, S. S. Thorgeirsson and J. K. Reddy, Expression of transcription factors and stem cell factor precedes hepatocyte differentiation in rat pancreas, *Gene Expr* 1996, **6**, 15-22.

105 M. L. Krakowski, M. R. Kritzik, E. M. Jones, T. Krahl, J. Lee, M. Arnush, D. Gu and N. Sarvetnick, Pancreatic expression of keratinocyte growth factor leads to differentiation of islet hepatocytes and proliferation of duct cells, *Am J Pathol* 1999, **154**, 683-91.

106 T. Yamaoka, K. Yoshino, T. Yamada, M. Yano, T. Matsui, T. Yamaguchi, M. Moritani, J. Hata, S. Noji and M Itakura, Transgenic expression of FGF8 and FGF10 induces transdifferentiation of pancreatic islet cells into hepatocytes and exocrine cells, *Biochem Biophys Res Commun* 2002, **292**, 138-43.

107 K. Wallace, P. A. Flecknell, A. D. Burt and M. C. Wright, Disrupted pancreatic exocrine differentiation and malabsorption in response to chronic elevated systemic glucocorticoid, *Am J Pathol* 2010, **177**, 1225-32.

108 P. A. Seymour, H. P. Shih, N. A. Patel, K. K. Freude, R. Xie, C. J. Lim and M. Sander, A Sox9/Fgf feed-forward loop maintains pancreatic organ identity, *Development* 2012, **139**, 3363-72.

109 C. N. Shen, J. R. Seckl, J. M. Slack and D. Tosh, Glucocorticoids suppress beta-cell development and induce hepatic metaplasia in embryonic pancreas, *Biochem J* 2003, **375**, 41-50.

110 S. Sumitran-Holgersson, G. Nowak, S. Thowfeequ, S. Begum, M. Joshi, M. Jaksch, A. Kjaeldgaard, C. Jorns, B. G. Ericzon and D. Tosh, Generation of hepatocyte-like cells from in vitro transdifferentiated human fetal pancreas, *Cell Transplant* 2009, **18**, 183-93.

111 F. Lang, C. Stournaras and I. Alesutan I, Regulation of transport across cell membranes by the serum- and glucocorticoid-inducible kinase SGK1, *Mol Membr Biol* 2014, **31**, 29-36.

112 P. Wulff, V. Vallon, D. Y. Huang, H. Völkl, F. Yu, K. Richter, M. Jansen, M. Schlünz, K. Klingel, J. Loffing, G. Kauselmann, M. R. Bösl, F. Lang and D. Kuhl, Impaired renal Na(+) retention in the sgk1-knockout mouse, *J Clin Invest* 2002, **110**, 1263-8.

113 M. Sobiesiak, E. Shumilina, R. S. Lam, F. Wölbing, N. Matzner, S. Kaesler, I. M. Zemtsova, A. Lupescu, N. Zahir, D. Kuhl, M. Schaller, T. Biedermann and F. Lang, Impaired mast cell activation in gene-targeted mice lacking the serum- and glucocorticoid-inducible kinase SGK1, *J Immunol* 2009, **183**, 4395-402.

114 M. N. Silverman and E. M. Sternberg, Glucocorticoid regulation of inflammation and its functional correlates: from HPA axis to glucocorticoid receptor dysfunction, *Ann N Y Acad Sci* 2012, **1261**, 55-63.

115 J. R. Flammer and I. Rogatsky, Minireview: Glucocorticoids in autoimmunity: unexpected targets and mechanisms, *Mol Endocrinol* 2011, **25**, 1075-86.

116 J. R. Seckl and M.C. Holmes, Mechanisms of disease: glucocorticoids, their placental metabolism and fetal 'programming' of adult pathophysiology, *Nat Clin Pract Endocrinol Metab* 2007, **3**, 479-88.

117 P. R. Provost, E. Boucher and Y. Tremblay, Glucocorticoid metabolism in the developing lung: adrenal-like synthesis pathway, *J Steroid Biochem Mol Biol* 2013, **138**, 72-80.

118 M. C. Wright and A. J. Paine, Induction of the cytochrome P450 3A subfamily correlates with the binding of inducers to a microsomal protein, *Biochem Biophys Res Commun* 1994, **201**, 273-279.

119 C. R. Padgham, C. C. Boyle, X. J. Wang, S. M. Raleigh, M. C. Wright and A. J. Paine, Alteration of transcription factor mRNAs during the isolation and culture of rat hepatocytes suggests the activation of a proliferative mode underlies their de-differentiation, *Biochem Biophys Res Commun* 1993, **197**, 599-605.

120 L. A. Scheving, M. C. Stevenson, X. Zhang and W. E. Russell, Cultured rat hepatocytes upregulate Akt and ERK in an ErbB-2-dependent manner, *Am J Physiol Gastrointest Liver Physiol* 2008, **295**, G322-31.

121 C. P. Hodgkinson, M. C. Wright and A. J. Paine, Fibronectin-mediated hepatocyte shape change reprograms cytochrome P450 2C11 gene expression via an integrin-signaled induction of ribonuclease activity, *Mol Pharmacol* 2000, **58**, 976-81.

122 N. S. Sharma, D. Nagrath and M. L. Yarmush, Adipocyte-derived basement membrane extract with biological activity: applications in hepatocyte functional augmentation in vitro, *FASEB J* 2010, **24**, 2364-74.

123 E. Kimoto, R. Walsky, H. Zhang, Y. A. Bi, K. M. Whalen, Y. S. Yang, C. Linder, Y. Xiao, K. Iseki, K. S. Fenner, A. F. El-Kattan and Y. Lai Y, Differential modulation of cytochrome P450 activity and the effect of 1-aminobenzotriazole on hepatic transport in sandwich-cultured human hepatocytes, *Drug Metab Dispos* 2012, **40**, 407-11.

124 A. Skardal, L. Smith, S. Bharadwaj, A. Atala, S. Soker and Y. Zhang, Tissue specific synthetic ECM hydrogels for 3-D in vitro maintenance of hepatocyte function, *Biomaterials* 2012, **33**, :4565-75.

125 M. J. Lima, H M. Docherty, Y. Chen and K. Docherty, Efficient differentiation of AR42J cells towards insulin-producing cells using pancreatic transcription factors in combination with growth factors, *Mol Cell Endocrinol* 2012, **358**, 69-80.

126 S. Marro, Z. P. Pang, N. Yang, M. C. Tsai, K. Qu, H. Y. Chang, T. C. Südhof and M. Wernig, Direct lineage conversion of terminally differentiated hepatocytes to functional neurons, *Cell Stem Cell* 2011, **9**, 374-82.

127 K. Woltjen, I. P. Michael, P. Mohseni, R. Desai, M. Mileikovsky, R. Hämäläinen, R. Cowling, W. Wang, P. Liu, M. Gertsenstein, K. Kaji, H. K. Sung and A. Nagy, piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells, *Nature* 2009, **458**, 766-70.

128 K. Kaji, K. Norrby, A. Paca, M. Mileikovsky, P. Mohseni and K. Woltjen, Virus-free induction of pluripotency and subsequent excision of reprogramming factors, *Nature* 2009, **458**, :771-5.

129 K. Wallace, A. D. Burt and M. C. Wright, Liver fibrosis, *Biochem J* 2008, **411**, 1-18.

130 C. J. Marek, K. Wallace, E. Durward, M. Koruth, V. Leel, L. J. Leiper and M. C. Wright, Low affinity glucocorticoid binding site ligands as potential anti-fibrogenics, *Comp Hepatol* 2009, **8**, 1.

131 J. G. Orr, V. Leel, G. A. Cameron, C. J. Marek, E. L. Haughton, L. J. Elrick, J. E. Trim, G. M. Hawksworth, A. P. Halestrap and M. C. Wright, Mechanism of action of the antifibrogenic compound gliotoxin in rat liver cells, *Hepatology* 2004, **40**, 232-42.

132 N. Eritja, C. Mirantes, D. Llobet, G. Masip, X. Matias-Guiu and X. Dolcet, ERα-mediated repression of pro-inflammatory cytokine expression by glucocorticoids reveals a crucial role for TNFα and IL1α in lumen formation and maintenance, *J Cell Sci* 2012, **125**, 1929-44.

133 M. L. Ruiz, J. P. Rigalli, A. Arias, S. Villanueva, C. Banchio, M. Vore, A. D. Mottino and V. A. Catania, Induction of hepatic multidrug resistance-associated protein 3 by ethynylestradiol is independent of cholestasis and mediated by estrogen receptor, *Drug Metab Dispos* 2013, **41**, 275-80.

134 G. G. Kuiper, E. Enmark, M. Pelto-Huikko, S. Nilsson and J. A. Gustafsson, Cloning of a novel receptor expressed in rat prostate and ovary, *Proc Natl Acad Sci U S A* 1996, **93**, 5925-30.

135 A. Axon, F. E. May, L. E. Gaughan, F. M. Williams, P. G. Blain and M. C. Wright, Tartrazine and sunset yellow are xenoestrogens in a new screening assay to identify modulators of human oestrogen receptor transcriptional activity, *Toxicology* 2012, **298**, 40-51.

136 C. Anacker, A. Cattaneo, K. Musaelyan, P. A. Zunszain, M. Horowitz, R. Molteni, A. Luoni, F. Calabrese, K. Tansey, M. Gennarelli, S. Thuret, J. Price, R. Uher, M. A. Riva and C. M. Pariante, Role for the kinase SGK1 in stress, depression, and glucocorticoid effects on hippocampal neurogenesis, *Proc Natl Acad Sci U S A* 2013, **110**, 8708-13.

137 J. Woudenberg, K. P. Rembacz, M. Hoekstra, A. Pellicoro, F. A. van den Heuvel, J. Heegsma, S. C. van Ijzendoorn, A. Holzinger, T. Imanaka, H. Moshage and K. N. Faber, Lipid rafts are essential for peroxisome biogenesis in HepG2 cells, *Hepatology* 2010, **52**, 623-33.

138 J. Lardon, S. De Breuck, I. Rooman, L. Van Lommel, M. Kruhøffer, T. Orntoft, F. Schuit and L. Bouwens, Plasticity in the adult rat pancreas: transdifferentiation of exocrine to hepatocyte-like cells in primary culture, *Hepatology* 2004, **39**, 1499-507.

139 S. Y. Wu C. C. Hsieh, R. R. Wu, J. Susanto, T. T. Liu, C. R. Shen, Y. Chen, C. C. Su, F. P. Chang, H. M. Chang, D. Tosh, C. N. Shen, Differentiation of pancreatic acinar cells to hepatocytes requires an intermediate cell type. *Gastroenterology* 2010, **138**, 2519-30.

140 R. M. Reynolds, Glucocorticoid excess and the developmental origins of disease: two decades of testing the hypothesis--2012 Curt Richter Award Winner, *Psychoneuroendocrinology* 2013, **38**, 1-11.

141 G. S. Ratra, W. A. Morgan, J. Mullervy, C. J. Powell and M. C. Wright, Methapyrilene hepatotoxicity is associated with oxidative stress, mitochondrial disfunction and is prevented by the Ca2+ channel blocker verapamil. *Toxicology* 1998, **130**, 79-93.

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REVIEW

Table 1. Hepatocyte phenotype in B-13/H cells, utility in hepatotoxicity studies – blood proteins, cell structure and metabolism.

Gene	Method of analysis	Comment
Blood proteins		
Albumin	ICC,[23] RT-PCR,[24] WB,[25,27] secretion ELISA[26]	Hepatocyte-specific, major blood protein.[30]
Transferrin	ICC,[23] RT-PCR,[24] WB[26]	Hepatocyte-enriched, also expressed by other tissues such as the brain and testis. Functions as an iron transporter.[31]
Transthyretin	ICC,[23] RT-PCR[24]	Hepatocyte-enriched, also expressed by chorioid plexus and retinal pigment epithelium. Functions as a thyroid hormone transporter.[32]
α1-antitrypsin	ICC[23]	Hepatocyte-enriched, but also expressed in monocytes, macrophages, pulmonary alveolar cells, and by intestinal and corneal epithelium . Protease inhibitor that functions to inhibit elastases as well as other proteases.[33]
A2-macroglobulin (αMG)	ICC,[25] RT-PCR,[25] WB[25]	Hepatocyte-enriched, also expressed in macrophages, fibroblasts, and adrenocortical cells. Acute phase response protein, broad spectrin protease inhibitor, functions to inhibit fibrinolysis.[34]
Haptoglobin (Hp)	ICC,[25] RT-PCR,[25] WB[25]	Hepatocyte-enriched, also expressed in skin, kidney and lung. Acute phase response protein, functions to bind and transport free haemoglobin.[35]
α-fetoprotein	ICC[23]	Foetal yolk sac and liver-specific (low levels in intestines), blood protein associated with foetus.[36] Expression in B-13/H cells not confirmed in other studies by RT-PCR.[24,29]
Apolipoprotein B	ICC[26]	Serves as ligand for the LDL receptor. Full length ApoB100 is expressed specifically by the liver.[37]
Cell structure		
Cytokeratin 8 (CK8)	ICC[23]	Structural protein associated with secretory epithelium such as hepatocytes.[38]
Metabolism		
Glucose 6 phosphatase	ICC,[23] RT-PCR [24]	Liver enriched, also expressed in, kidney, intestine, and pancreatic islets .[39]
Tyrosine aminotransferase	RT-PCR [24]	Liver –specific. This gene is transcriptionally regulated by the GR.[40]
Arginosuccinate synthase	RT-PCR [24]	Urea cycle enzyme, expressed predominantly in the liver but is also expressed in most other tissues such as fibroblasts, kidney, heart, brain, muscle, pancreas, and red blood cells.[41]
Carbamyl synthetase (CPSI)	RT-PCR,[24] WB,[24,26,27] ICC[27]	CPSI is a urea cycle enzyme expressed in rat liver throughout much of the lobule but not in proximal centrilobular hepatocytes.[42,43] B-13/H cells positive for CPSI were negative for glutamine synthase by ICC.[28]
Glutamine synthase (GS)	ICC[28]	Ammonia removing enzyme, GS is expressed in rat liver only in the in proximal centrilobular hepatocytes.[42,43] B-13/H cells positive for GS were negative for CPSI.[28]
Ornithine transcarboxylase	RT-PCR[25]	Urea cycle enzyme.[43]
Glucokinase	ICC[28]	Expressed in liver and other tissues.[44]

ICC, immunocytochemistry; RT-PCR, RT-PCR determination; WB, Western blotting; ELISA, enzyme linked immunosorbent assay-based determination.

Table 2. Hepatocyte phenotype in B-13/H cells, utility in hepatotoxicity studies – xenobiotic metabolism and related.

Gene	Method of analysis	Comment
Xenobiotic metabolism and related		
CYP450 reductase	WB[45,26]	Essential for functional microsomal cytochrome P450 activity.[49] Required for toxic effects of many drugs and chemicals.[129]
Total CYP450	SPEC[45,27]	Major xenobiotic metabolising gene superfamily.[50] Reduced CO versus reduced spectra peak at 450nm. It is possible to generate cells with similar levels to rat hepatocytes detectable.[27] Required for toxic effects of many drugs and chemicals.[129]
Cytochrome b5	SPEC	Unpublished.
Aryl hydrocarbon receptor (AhR)	RT-PCR[46]	Receptor for polyaromatic hydrocarbon xenobiotics – regulates induction of CYP1 genes.[51]
Aryl hydrocarbon receptor nuclear translocator (AhRNT)	RT-PCR[46]	Heterodimerizes with AhR , essential for effective AhR-mediated transcriptional function.[51]
CYP1A1	RT-PCR,[46] WB,[46] FUNC[46].	Expression induced by AhR activator β-naphthoflavone.[46] See also Fig. 6. Required to generate genotoxic products from many polyaromatic hydrocarbons such as benz(α)pyrene.[46]
CYP1A2	Absent - RT-PCR,[46] absent – WB,[46] absent – FUNC[46]	All alleles appear disrupted in cell line.[46] Expression of human CYP1A2 mediates PhIP activation to a genotoxic product.[46]
Constitutive androstane receptor (CAR)	RT-PCR[46]	Transcriptional regulator of CYP2B induction response .[52]
CYP2B1	RT-PCR,[46] absent – WB,[46] present WB,[26] ICC,[28] absent – FUNC[46]	CAR activators result in induction in mRNA expression, but translation is lacking.[46] See also Fig. 6. Note, CYP2B1 is not constitutively significantly expressed in rat hepatocytes, only after administration of inducers .[53]
CYP2B2	Absent - RT-PCR,[46] ICC[29]	CYP2B2 is constitutively expressed at only low levels in rat hepatocytes.[53]
CYP2A1/2	WB[45]	
CYP2C6	RT-PCR, [24,46] WB[46]	An adult hepatic gene expressed at high and similar levels in both male and female rats.[54]
CYP2C11	RT-PCR, [45,46] WB,[45,46] FUNC[45]	CYP2C11 is the major (male-specific) adult hepatic cytochrome P450. Expression is liver-specific.[55] Metabolises methapyrilene to an hepatotoxic product,[141] also observed in B-13/H, but not in B-13 cells.[46]
CYP2C12	Low - RT-PCR[46]	CYP2C12 is the major (female-specific) adult hepatic cytochrome P450, Expression is liver-specific.[55] Levels of mRNA in B-13/H cells are less than 1% of the levels seen in female rat liver.[46]
CYP2C13	RT-PCR[47]	Male-specific isoform, specific to liver.[56]
CYP2D1	RT-PCR[47]	
CYP2E1	RT-PCR,[24,45,46], WB,[24,26,45,48] ICC,[26,46] FUNC[48]	Paracetamol is activated to a toxic metabolite.[45]
Pregnane X receptor (PXR)	RT-PCR,[46] FUNC[46]	Receptor for drugs and xenobiotics – regulates induction of CYP3A genes.[52] Receptor regulates inducible expression of CYP3A sub-family – the orthologous sub-family in humans constitutes the predominant drug metabolising sub-family

		responsible for the metabolism of approx. half of all drugs in man.[50]
CYP3A1	RT-PCR, [46] WB,[45,46,26] ICC [29]	Inducible isoform.[46] See also Fig. 6.
CYP3A2	RT-PCR,[46] WB,[45] FUNC[45]	Adult liver-specific isoform, male predominant expression.[57]
CYP3A9	RT-PCR[46]	
Peroxisome proliferator activated receptor α (PPARα)	RT-PCR[47]	Receptor for peroxisome proliferator drugs such as fibrates – regulates induction of CYP4A genes.58
CYP4A1	Absent - RT-PCR,[47] absent – WB[47]	Many CYP4A genes appear to be disrupted in B-13 cells – no induction of proteins - see Fig. 6.
CYP4A2	Absent - RT-PCR[47]	
CYP4A3	Absent - RT-PCR[47]	
Farnesoid X receptor	Low expression	Unpublished. Receptor for bile acids – regulator of bile acid synthesis.[59]
CYP7A1	WB[26]	
Liver X receptor	RT-PCR	Both LXRα and LXRβ are expressed in B-13/H cells (unpublished). Transcriptionally functional in B-13/H cells (unpublished). Receptor for oxysterols.
Phase II enzyme systems		
Sulfotransferases (SULTs)		5/14 genes expressed at mRNA level.[46] Activates estragole to a genotoxic product in B-13/H cells.[79]
SULT1A1	ICC,[23,29] RT-PCR[46]	Substrate is 4 nitrophenol.[60]
SULT1B1	RT-PCR[46]	Substrate is 4 nitrophenol.[60]
SUL2B1	RT-PCR[46]	Substrates are steroids and bile acids.[60]
SULT4A1	RT-PCR[46]	
SULT5A1	RT-PCR[46]	
UDP glucuronyltransferases (UGTs)		5/8 hepatic genes expressed at mRNA level.[46]
UGT1A1	RT-PCR[46]	Main conjugating enzyme of bilirubin and drugs such as the anticancer drug irinotecan. Other substrates include estradiol.[61]
UGT1A5	RT-PCR[46]	Transiently elevated in immature rat liver.[62]
UGT1A6	ICC[23]	4 nitrophenol is a substrate.[61]
UGT1A7	n/d	Paracetamol is a substrate. However, expression is low in liver, expressed high in gut.[63]
UGT1A8	RT-PCR[46]	
UGT2B1	RT-PCR[46]	Substrates include morphine and testosterone.[64]
UGT2B3	RT-PCR[46]	

Glutathione transferases (GSTs)	RT-PCR[46]	15/21 genes expressed at mRNA level..[46] Catalyze conjugation reactions between reduced glutathione (GSH) and electrophilic substrates.[65]

ICC, immunocytochemistry; RT-PCR, RT-PCR determination; WB, Western blotting; FUNC, functional assay; n/d, not determined; SPEC, spectrophotometric determination..

Table 3. Hepatocyte phenotype in B-13/H cells, utility in hepatotoxicity studies – endobiotic and xenobiotic transporters.

Gene	Method of analysis	Comment
Transporters (endobiotic/xenobiotic related)		
Abcb11 (BSEP)	Low - RT-PCR,[46] FUNC,[46] ICC[28]	Canalicular-localised transporter, mediates bile acid efflux.[66] Inhibited by troglitazone.[67]
Abcb1b (MDR1/pglycoportein1)	RT-PCR,[46] FUNC[46]	Canalicular-localised transporter, mediates efflux of digoxin, talinolol, quinidine and fexofenadine in man .[68] Verapamil is an inhibitor in man.[68] Higher mRNA levels in B-13/H than intact rat liver.[46]
Abcb4 (MDR2/pglycoportein3)	RT-PCR[46]	MDR2 is the rodent orthologue of human MDR3. Canalicular-localised transporter that mediates the biliary excretion of phospholipids.[69] Similar mRNA levels in B-13/H to intact rat liver.[46]
Abcc1 (MRP1)	RT-PCR,[46] FUNC[46]	Mediates efflux of leukotrienes, oestrogen conjugates, vincritine, etoposide.[70] Normal human hepatocytes lack detectable amounts of MRP1.[71] Basolateral-localised in normal tissues.[71] Higher mRNA levels in B-13/H than intact rat liver.[46]
Abcc2 (MRP2)	Low - RT-PCR,[46] FUNC[46]	Canalicular-localised transporter, mediates efflux of amphiphilic conjugates, such as bilirubin glucuronides, glutathione conjugates, and sulfate conjugates.[72]
Abcc3 (MRP3)	RT-PCR,[46] FUNC[46]	Basolateral-localised transporter,[73] mediates efflux of glutathione, sulphate and glucuronide conjugates, such as estradiol-17beta-glucuronide, bilirubin-glucuronides, and etoposide-glucuronide.[73]Higher mRNA levels in B-13/H than intact rat liver.[46]
Abcc4 (MRP4)	RT-PCR,[46] FUNC[46]	Basolateral-localised transporter, possibly mediates transport of cyclic nucleotides such as cAMP);[74] low affinity bile salt export; conjugated steroids and prostanoids.[73] Higher mRNA levels in B-13/H than intact rat liver.[46]
Abcc5 (MRP5)	RT-PCR,[46] FUNC,[46]	Basolateral-localised transporter, possibly mediates transport of cyclic nucleotides such as cGMP[74] although this has been questioned.[73,75] Higher mRNA levels in B-13/H than intact rat liver.[46]
Abcc6 (MRP6)	RT-PCR,[46] FUNC[46]	Basolateral-localised transporter, predominantly expressed in the plasma membrane of the hepatocyte (and to a lesser extent in the kidney).[76] Mutations in the gene cause a rare, recessive genetic disease, pseudoxanthoma elasticum.[76] Similar mRNA levels in B-13/H to intact rat liver.[46]
Abcg2 (BCRP)	RT-PCR,[46] FUNC[46]	Canalicular-localised transporter, mediates efflux transport of many chemotherapeutics, and involved in haem, folate and uric acid homeostasis.[77] This transporter is often associated with stem/progenitor cells.[77] Similar mRNA levels in B-13/H to intact rat liver.[46]
Slc10a1 (NTCP)	Low - RT-PCR[46]	Basolateral -localised transporter, mediates uptake of bile salts, sulfated compounds, thyroid hormones, drugs and toxins.[78] Protein levels are absent in B-13/H and many other cells in culture, limiting their usefulness in toxicity screening studies.[79]
Slc22a1 (OCT1)	Low - RT-PCR[46]	Basolateral -localised transporter, mediates uptake of organic cations.[80] Expressed at high levels in liver.[81]
Slc22a2 (OCT2)	RT-PCR[46]	Basolateral -localised transporter, mediates uptake of organic cations.[81] Expressed at high levels in kidney.[81] Similar mRNA levels in B-13/H to intact rat liver.[46]
Slc22a6 (OAT1)	RT-PCR[46]	Expressed predominantly in the kidneys and localized exclusively to the basolateral membrane of proximal tubular cells. Mediates the uptake of various small, negatively charged molecules, such as endogenous metabolites, toxicants, and numerous clinically used therapeutics - functions as an antiporter exchanging intracellular α-ketoglutarate against extracellular organic anions and drugs.[82] Similar mRNA levels in B-13/H to intact rat liver.[46]

Slc22a7(OAT2)	Low - RT-PCR[46]	Expressed in liver and kidney. Apical renal transporter in rat.[82] It is unclear why OAT2 in humans and Oat2 in rodents show a basolateral versus apical localization in proximal tubules. The presumed localization of OAT2/Oat2 in the sinusoidal membrane of hepatocytes awaits an experimental proof .[82]
Slc22a8 (OAT3)	Low - RT-PCR[46]	Basolateral renal transporter in rat, also expressed in liver.[82]
Slc22a9 (OAT5)	RT-PCR[46]	Apical renal transporter in rat.[82] Similar mRNA levels in B-13/H to intact rat liver.[46]
Slco1a5 (OATP3)	RT-PCR[46]	Basolateral-localised transporter, primarily expressed in the choroid plexus in rat.[83] Similar mRNA levels in B-13/H to intact rat liver.[46]
Slco1a6 (OATP5)	RT-PCR[46]	Basolateral-localised transporter, primarily expressed in the kidney in rat.[83] Similar mRNA levels in B-13/H to intact rat liver.[46]
Slco2b1 (MOAT)	Low - RT-PCR[46]	Transporter for prostaglandins, leukotrienes and taurocholate.[84]

ICC, immunocytochemistry; RT-PCR, RT-PCR determination; WB, Western blotting.

Table 4. Reports for evidence for hepatocyte formation in the pancreas in vivo.

Condition/treatment	Species	Comments
Pancreatic liver regeneration[93]	Hamster	Pancreatic regeneration was induced by maintaining animals on a methionine-deficient and i.p. treatment with 500mg/kg bw/day DL-ethionine for 8 days. On the ninth day, animals were returned to a full amino acid diet and given a single i.p. injection of 800 mg/kg bw methionine. Morphological alterations of cells in pancreas observed.
Pancreatic liver regeneration / methyl clofenapate[94]	Hamster	Pancreatic regeneration was induced by maintaining animals on a methionine-deficient and i.p. treatment with 500mg/kg bw/day DL-ethionine for 8 days. On the ninth day, animals were returned to a full amino acid diet and given a single i.p. injection of 800 mg/kg bw methionine. Treatment with the peroxisome proliferator methyl clofenapate resulted in peroxisome proliferationand increases in the expression of markers of peroxisome proliferation in the pancreas. Cytochemical localization of catalase and immunofluorescence localization of enoyl-CoA hydratase suggested peroxisome proliferation occurred in pancreatic hepatocyte-like cells and not adjacent acinar, duct and islet cells.
Ciprofibrate[95]	Rat	Animals were treated with the peroxisome proliferator ciprofibrate at 10mg/kg bw/day for 60-72 weeks. Primarily a morphometric study demonstrating appearance of hepatocyte like cells localized adjacent to islets and penetrating into exocrine tissue.
Copper depletion/repletion[96]	Rat	Animals were fed a copper-deficient diet containing 0.6% D-penicillamine for 8-10 weeks, followed by copper repletion. Morphometric study combined with immunohistochemical staining for albumin and catalase (in pancreatic hepatocytes) and absence of pancreatic enzymes and hormones. Ciprofibrate also shown to induce peroxisome proliferation in the pancreatic hepatocytes.
Copper depletion/repletion[97]	Rat	Pancreatic hepatocytes shown to express CPSI and urate oxidase – genes expressed highly in hepatocytes.
Copper depletion/repletion[98]	Rat	Animals were maintained on a copper-deficient diet containing the copper-chelating agent triethylenetetramine tetrahydrochloride at 0.6% w/w for 7-9 weeks and then returned to normal rat chow. This resulted in low mortality (10%); pancreatic depletion of acinar cells and the development of multiple foci of hepatocytes in all 100% of rats - in some animals, liver cells occupied more than 60% of pancreatic volume within 6-8 weeks of recovery. Albumin mRNA expression confirmed in hepatocyte containing pancreata.
Cadmium chloride[99]	Rat	Repeated sub cutaneous injections of cadmium chloride resulted in the appearance of pancreatic hepatocytes based on rat albumin and connexin 32 expression.
Dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin, TCDD) treatment[100]	Hamster	Two or 6 intraperitoneal injections of 100μg/kg bw at 4 week intervals induced pancreatic hepatocytes in 75% and 89% of the animals respectively.
Copper depletion/repletion[101]	Rat	Pancreatic hepatocytes appear in both male and female pancreata in this model. Male pancreatic hepatocytes synthesize in all cells, the male liver (centrilobular-specific) alpha 2u-globulin under androgenic regulation. Expression was reduced after orchiectomy.
Copper depletion/repletion[102]	Rat	CPSI and glutamine synthetase are co-expressed in pancreatic hepatocytes, in contrast to the liver where they show marked demarcation across the hepatic lobule. Expression confirmed at mRNA level
Copper depletion/repletion[103]	Rats	Carcinogens that cause nucleolar segregation in parenchymal cells of liver, also have the same effect in pancreatic hepatocytes. Dietary administration of 2-acetylaminofluorene (0.025%) for 12 to 32 weeks also led to the development of glutathione S-transferase-P-positive pancreatic hepatocytes in the pancreas. Oval cell proliferation was observed in close association with pancreatic hepatocytes.
Copper depletion/repletion[104]	Rats	Expression of liver-enriched transcription factors during oval cell proliferation in the pancreas preceded the expression of albumin mRNA and subsequent differentiation of hepatocyte phenotype
Genetic model - ectopic expression of keratinocyte growth factor[105]	Mouse	Insulin promoter-regulated KGF resulted in the appearance of hepatocytes within the islets in the pancreas.

Genetic model – ectopic expression of FGF8[106]	Mouse	Glucagon promoter-regulated expression of FGF8 resulted in the appearance of hepatocyte-like cells in the periphery of pancreatic islets.
Dexamethasone treatment[24]	Rat	Administration of dexamethasone i.p. resulted in the appearance of occasional hepatocytes in the pancreas after 3 weeks.
Genetic model of elevated systemic glucocorticoid levels (Cushing's disease)[107]	Mouse	Pancreatic hepatocytes appear by 21 weeks of age as determined by transcript analysis, WB and ICC. Adrenalectomy prevents appearance of pancreatic hepatocytes.
Genetic model – ablation of Sox9[108]	Mouse	Pancreatic progenitor-specific ablation of Sox9 during early pancreas development caused pancreas-to-liver cell fate conversion
Resected human pancreas from patients maintained on long term systemic glucocorticoid therapy[90]	Human	Pancreatic hepatocytes observed in resected human pancreas from patients maintained on long term systemic glucocorticoid therapy, as determined by transcript analysis, WB and ICC.

Note that the isolation and culture of rat or mouse acinar cells in vitro and treatment with glucocorticoid is also capable of inducing the expression of genes associated with the hepatocyte,[138,139] although the levels of expression are not comparable to those seen in B-13/H cells.

Figure 1. Origin of the AR42J and AR42J-B13 (B-13) cell lines. Upper panels, light micrographs of the indicated cell line, lower panel, fluorescence immunocytochemical staining as outlined,[48] note cytoplasmic staining of the CYP2E1, DAPI staining DNA and identifies the nucleus.

Figure 2. Localisation of putative synaptophysin positive progenitor cells in rat liver and pancreas. A, schematic diagram of pancreas and liver tissue and potential analogous position of putative progenitors in the respective tissues. **B**, immunohistochemical staining for syntaptophysin and DAB detection in rat pancreas (left panels) and liver (right panels) stained essentially as outlined.[130] Dotted panel is expanded in lower panel; d, duct, pv, perivenous region; p, putative progenitor.

Figure 3. Isolation and culture of putative progenitor cells from rat liver results in generation of duct cells and fibroblasts. Putative progenitor cells were isolated from pronase digestion of the bilary tree remaining after a two-step collagenase digestion of rat liver[131] **A**, Light micropgraphs (upper panels) of cell types observed after 3 days of culture. Lower panels, immunocytochemical staining for progenitor (OV-6), ductal (CK-19 and fibroblast (vimentin) markers[129] in the morphologically distinct cell types after 3 days of culture, stained essentially as described .[48] B, RT-PCR analysis for the indicated transcript essentially as described.[24,48]

Figure 4. Oestrogens do not affect B-13 cell differentiation via the ER or GR. Recent work has demonstrated that B-13 cells are capable of differentiating into ductal-like cells[87] and that an alternative nuclear receptor – the oestrogen receptor-α (ERα) – mediates the effects of glucocorticoids in lumen-forming cells (such as the progenitor cells of the liver, which are bi-potential and capable of forming hepatocytes or the lumen-forming cholangiocytes).[132] **A**, demonstrates that the mRNA for ERα as well as the related ER gene ERβ are not detectable in B-13 cells in contrast to rat hepatocytes (which are known to express ERα[133]) and uterus and the ovary (which has previously been reported to express the ERβ[134]). RT-PCR analysis for the expression of ERα and ERβ mRNA transcripts in 4 separate B-13 cell cultures, after 30 cycles essentially as described.[24,48] **B**, transfection of an oestrogen receptor-responsive reporter gene construct (ERE_3-pGL3promoter)[135] into B-13 cells and treatment with either DEX or the natural endogenous oestrogen receptor activating ligand 17β oestradiol (E2) resulted in significant ERE_3-pGL3promoter reporter gene expression in response to DEX[*]. However, only the GR antagonist RU486 - and not two oestrogen receptor antagonists (tamoxifen or ICI182780) significantly inhibited DEX-dependent increases in ERE_3-pGL3promoter reporter gene expression[$]. These data therefore suggest that DEX-dependent activation of the GR is responsible for the increase in ERE_3-pGL3promoter reporter gene expression, likely due to high GR expression in B-13 cells. This is supported by alterations in phenotype (**C**) or expression of hepatic markers (**D**) as determined by Western blotting.[24,48]

Figure 5. Over-expression of SGK1F induces a trans-differentiation of B-13 cells to B-13/H cells without the addition of glucocorticoid. B-13 cells were infected with an adenovirus encoding the human SGK1F isoform (AdV-SGK1F) which has been previously shown to direct B-13 differentiation into B-13/H cell,[92] or a control adenovirus encoding NTCP (AdV-control) and morphology examined after 3 days (**A**) or cells harvested at day 5 for Western blotting (**B**) for the indicated protein.[24,48] Where indicated, cells were also treated with dexamethasone (DEX), the GR antagonist RU486 or the SGK1 antagonist GSK 650394 (GSK).[136] For more details on variant SGK1 isoforms and methodology, see Wallace et al, including supplementary data.[92] **C**, Schematic diagram illustrating the cross talk between the GR, SGK1 and WNT signalling pathways operating in B-13 cells.

Figure 6. Induction of hepatic cytochrome P450s in B-13, B-13/H and rat hepatocytes in vitro. B-13, B-13/H and rat hepatocytes in culture were treated with established cytochrome P450 inducersdaily for 3 days (androstenol, 5μM; β-naphthoflavone [β-NF], 20μM; phenobarbitone [PB], 1mM; 1,4-Bis-[2-(3,5-dichloropyridyloxy)]benzene, 3,3′,5,5′-Tetrachloro-1,4-bis(pyridyloxy)benzene [TCPOBOP], 1.5μM; dexamethasone [DEX], 10μM; pregnenolone 16α carbonitrile [PCN], 2μM or bezafibrate, 250μM as outlined,[46] followed by Western blotting for the indicated protein. [24,48]

Figure 7. PPARα-dependent peroxisomal proliferation, but not CYP4A induction, is functional in B-13/H cells. DNA from B-13 cells was subjected to mate-pair library construction and SOLiD Whole Genome sequencing and analysed as outlined,[46] **A**, illustrates significant insertions/deletions in the CYP4A locus which

likely explains lack of constitutive and bezafibrate-inducible expression of CYP4A genes as determined by RT-PCR (**B**), qRT-PCR for CYP4A1 mRNA; primary hepatocytes and B-13/H cells were treated for 3 days daily or over 14 days (B-13/H cells only) with vehicle (0.1% (v/v) DMSO) or 250μM bezafibrate in DMSO from a 1000-fold molar concentrated stock (**C**), bars are the mean and standard deviation of 3 independent determinations from a single experiment, typical of at least 3 separate experiments, *indicates significant difference compared to the indicated group at $p<0.05$. and Western blotting (**D**), cells were treated for 3 days as outlined for B and harvested 24 hours after the last treatment, results typical of 3 separate experiments. All methodologies have been previously published.[24,46,48] **E**, peroxisomal proliferation in response to bezafibrate treatment in B-13/H cells. B-13/H cells were treated with vehicle (0.1% (v/v) DMSO) or 250μM bezafibrate for 14 days. Following treatment cells were immunostained for the peroxisomal memberane protein 70 (PMP70) marker[137] essentially as previously described.[46] The cells were then counterstained with DAPI. Cells were photographed using a Zeiss fluorescence microscope and staining quantified using ImageJ particle counter. Results were normalised to cell number. Results are the mean and SD of 5 fields of view from the same experiment, typical of at least 3 separate experiments. Scale bar indicates 48μm. *significantly different to vehicle control at $p<0.05$.

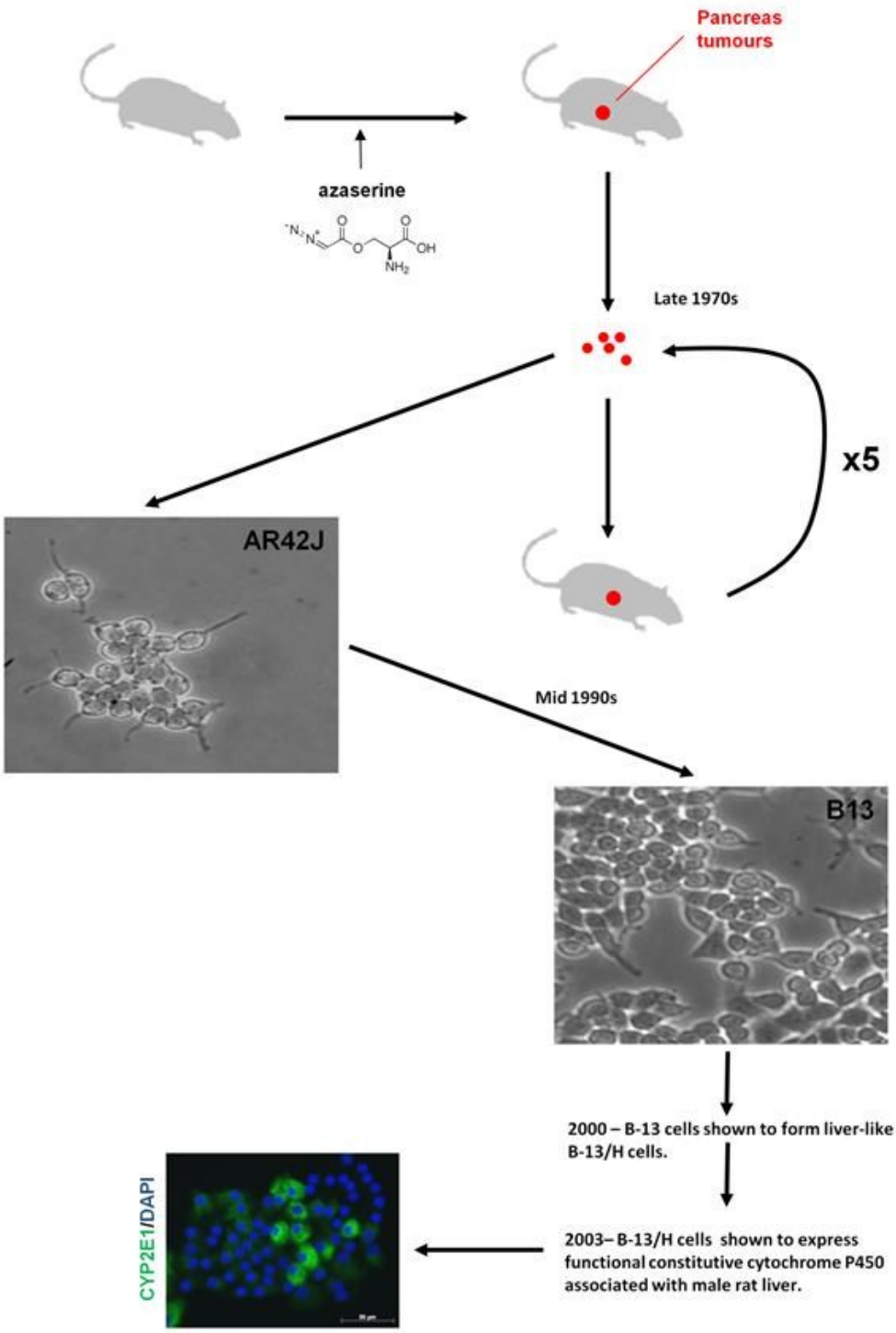
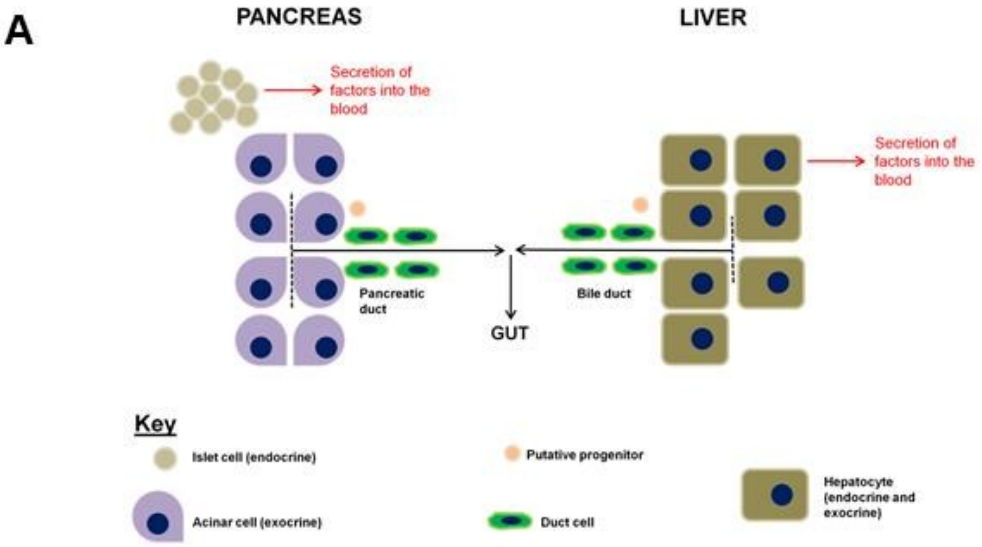


Figure 1



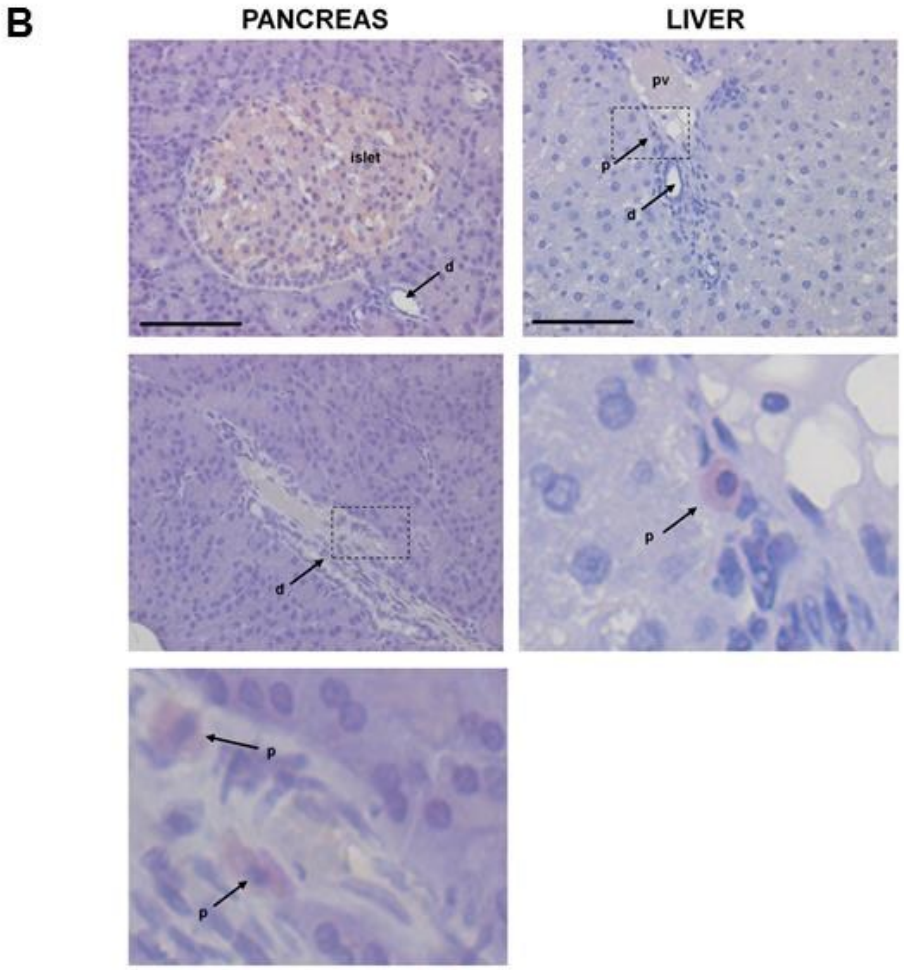


Figure 2

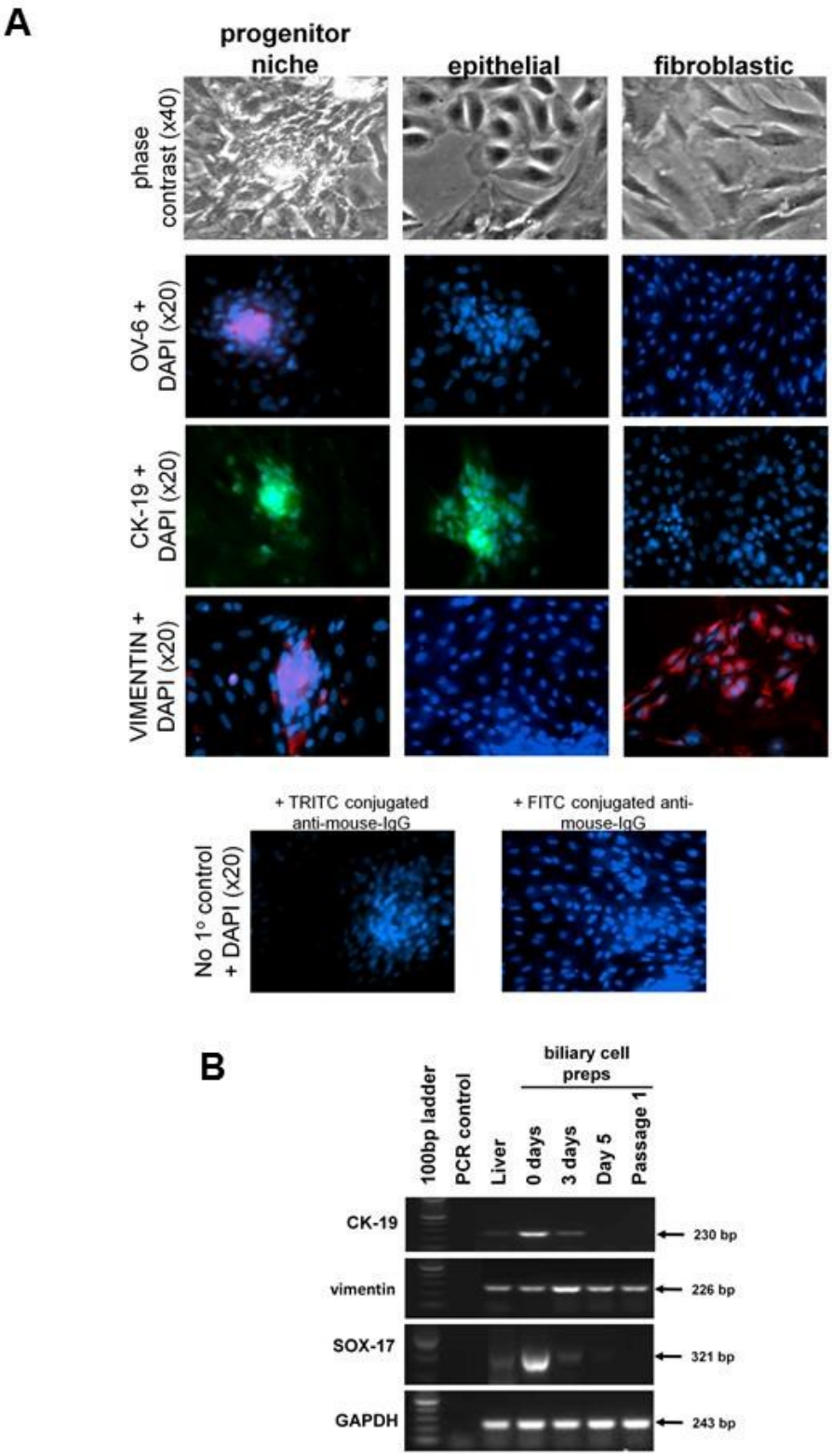


Figure 3

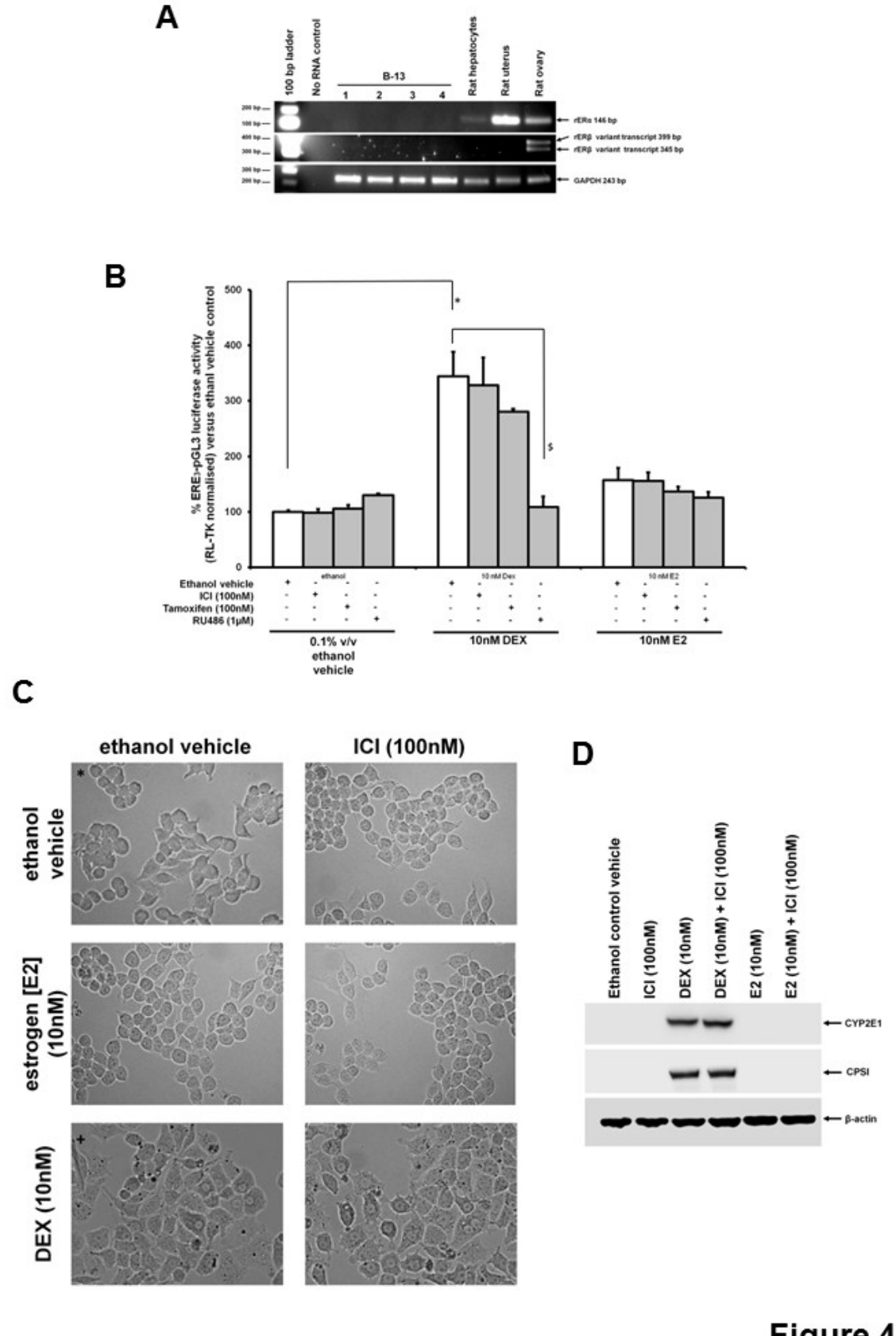


Figure 4

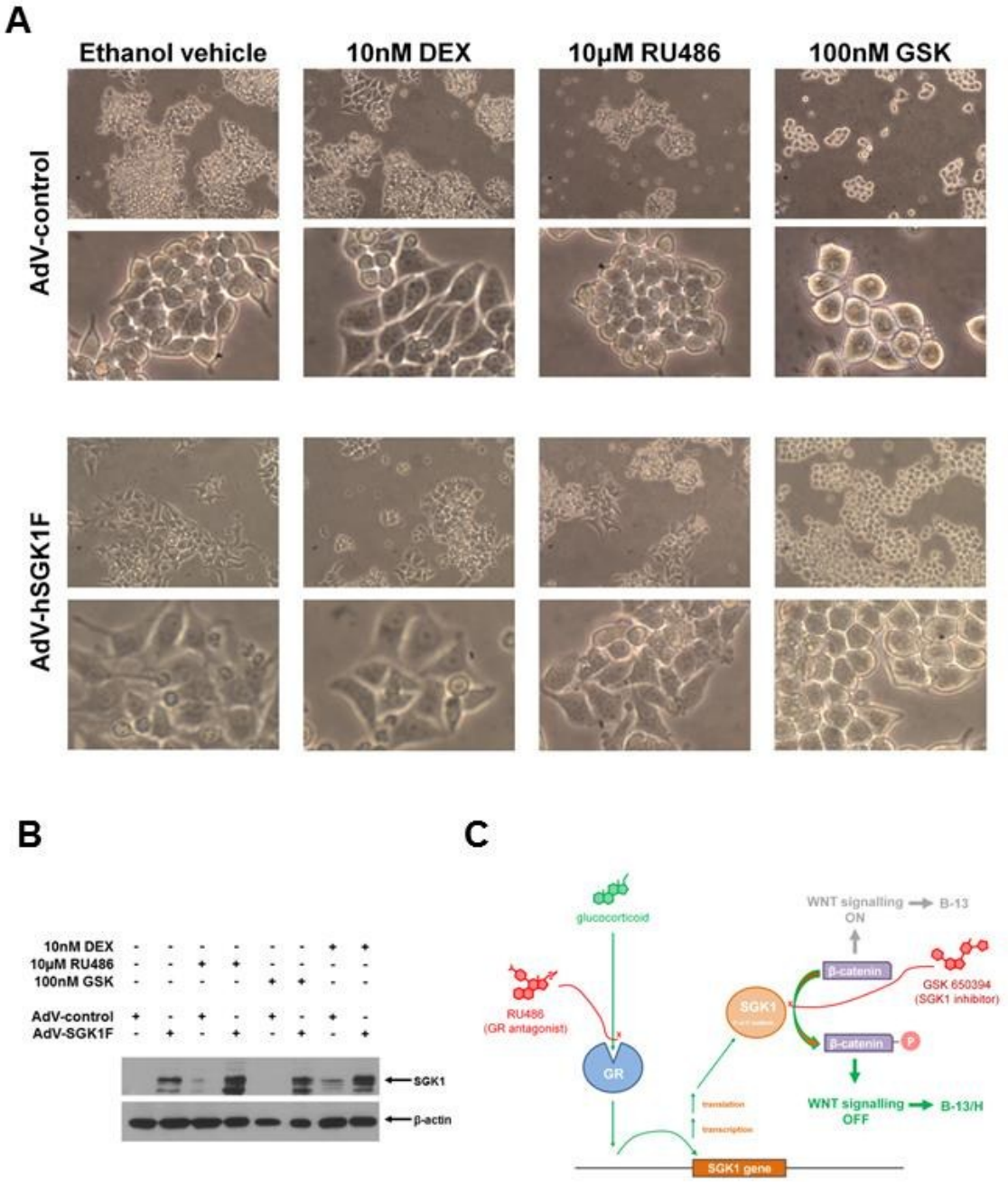


Figure 5

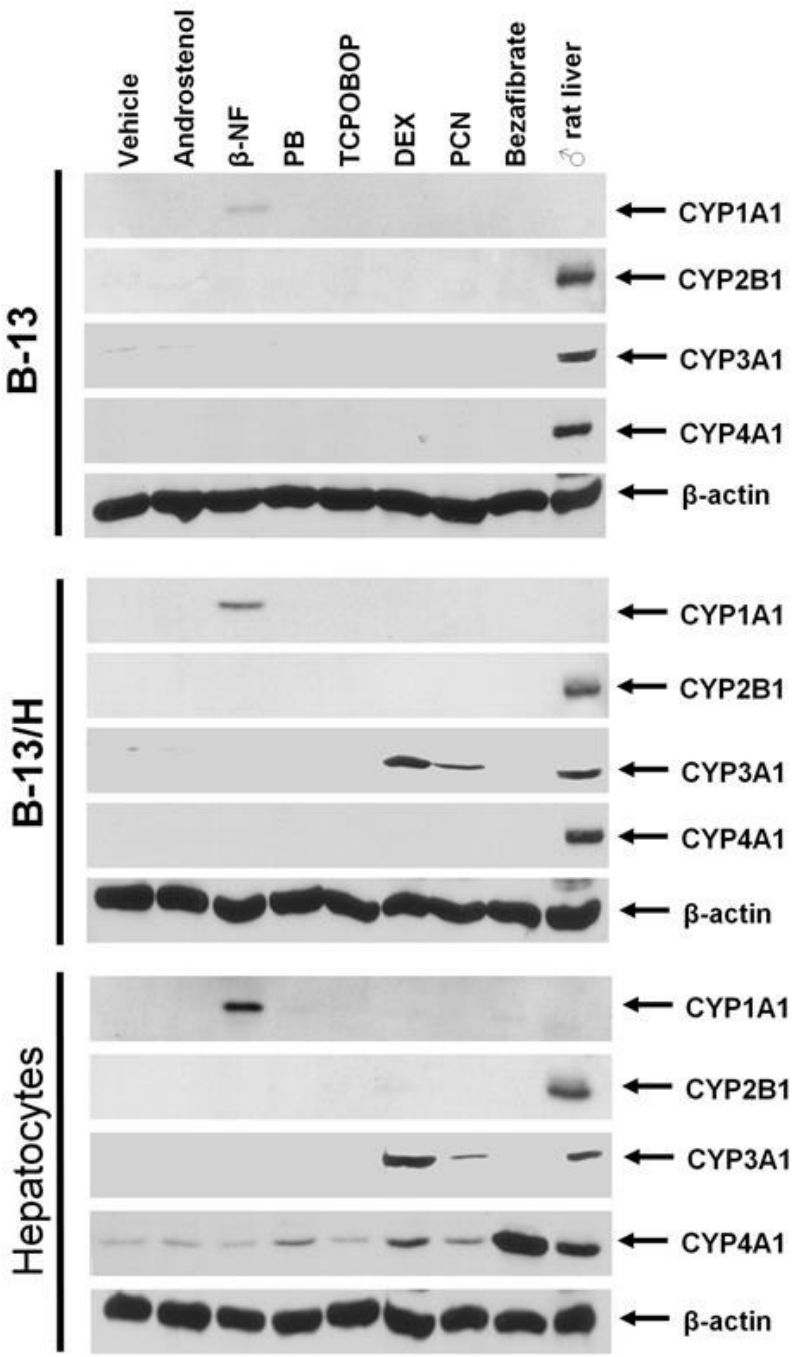


Figure 6

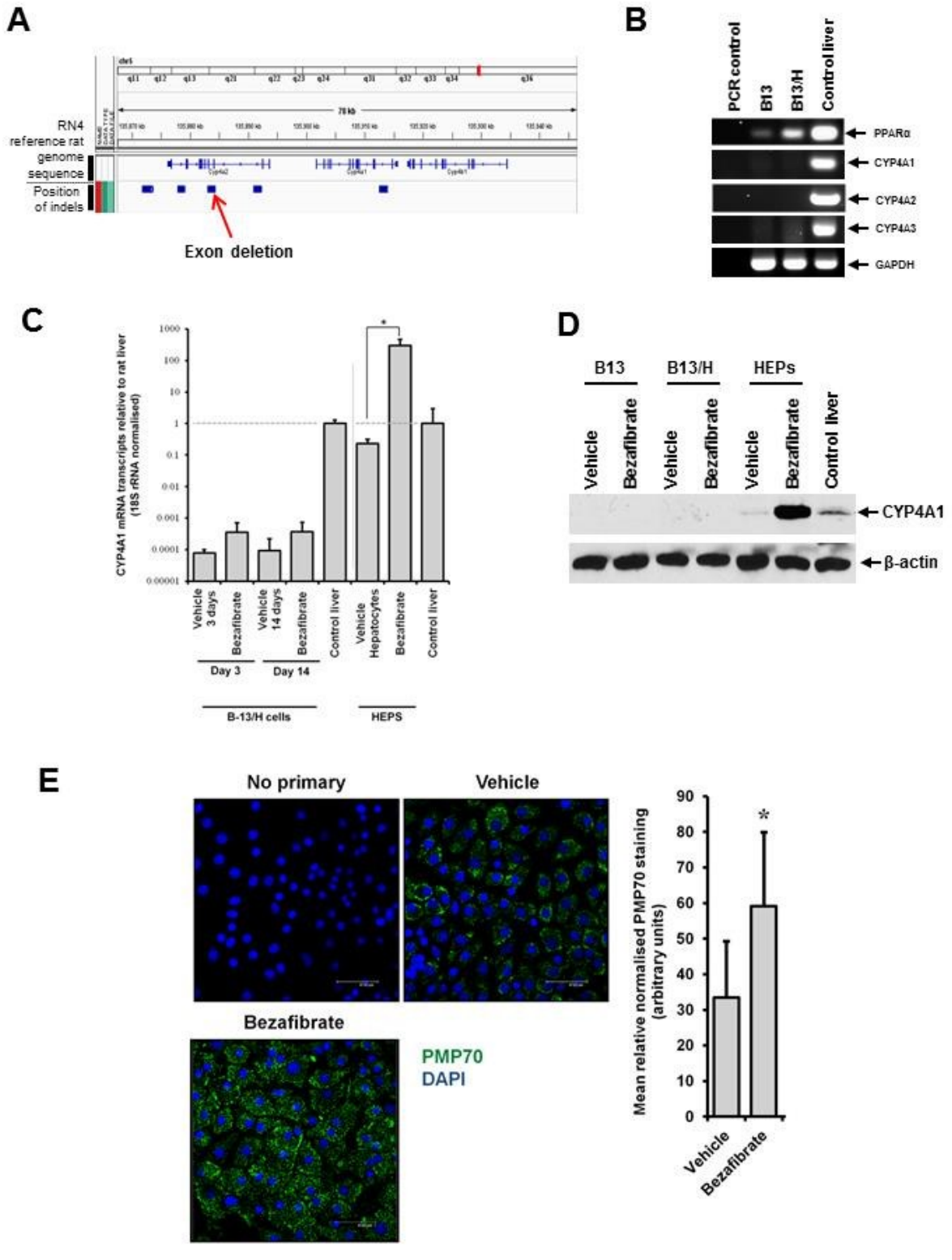


Figure 7